AUG 4 1930
Medical Lib.

BULLETIN

OF THE

JOHNS HOPKINS HOSPITAL

(THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL)

(SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY)

Vol. XLVII, No. 2 AUGUST, 1930

Contents

The Response of Plasma Water and Electrolytes to Elevation of Body Temperature. RUSTIN MCINTOSH, M.D., LASLO KAJDI, M.D. AND DOROTHY MEEKER, A.B..... 61

Response of Blood Guanidine Base Concentration in Normal Individuals and in Patients with Liver Injury to the Ingestion of Methyl Guanidine Sulphate. READ MCLANE ELLSWORTH 106

Disappearance of Diabetes Mellitus During the Development of Cirrhosis of the Liver. JAMES BORDLEY III..... 113

PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS

BALTIMORE, MARYLAND

Made in United States of America

Entered as second-class matter at the Baltimore, Md., Postoffice. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

3 IMPORTANT AIDS TO PHYSICIANS AND SURGEONS

For Respiratory Diseases

DISULPHAMIN

Orto-oxibenzoyl-sulphon-nucleino-formol-sodium-tetradimethylamino-antipyrin-bicamphorated

A Reliable Oxytocic

THYMOPHYSIN

(Tomasváry)

A Valuable Hemostatic

STRYPHNON

(Meyer & Allrecht)

Please send literature on items checked:

DISULPHAMIN ☐ THYMOPHYSIN ☐ STRYPHNON ☐

AMERICAN BIO-CHEMICAL LABORATORIES, INC.

27 Cleveland Place New York

Sole Agents for Canada NATIONAL DRUG AND CHEMICAL COMPANY *of Canada, Ltd, Montreal*

CORRECTION

Volume XLVI, No. 1, January issue. On page 90, Mr. *R. T. Nicoll* should read Mr. R. T. NICHOL.

THE RESPONSE OF PLASMA WATER AND ELECTROLYTES TO ELEVATION OF BODY TEMPERATURE[1]

RUSTIN McINTOSH, M.D., LASLO KAJDI, M.D. AND
DOROTHY MEEKER, A.B.

From the Harriet Lane Home of the Johns Hopkins Hospital and the Department of Pediatrics of the Johns Hopkins University School of Medicine

In the course of certain investigations in this laboratory in which the serum electrolyte concentration of rabbits was studied before and after the parenteral injection of vaccines, a depressing action of the vaccine on the serum concentration of total fixed base was brought out. The possibility arose that this might be the result of blood dilution from an influx of fluid, of relatively low electrolyte concentration, from reservoirs of intercellular fluid; and this led to an investigation of simultaneous changes in circulating blood volume and serum electrolyte concentration. Although the literature of physiology and clinical medicine affords numerous reports of studies of either of these functions alone in response to various bodily alterations such as infection, poisoning, and the parenteral injection of fluids, or alterations of environment such as a raising or lowering of the temperature of the atmosphere, the simultaneous determination of the two together appears to have been attempted only by Soule, Buckman, and Darrow (1) in fever of infectious origin.

Using the dye method of Keith, Rowntree and Geraghty (2) for plasma volume and the method of Smith (3) for chlorides, these authors made simultaneous determinations of these two factors in several infants and children suffering from a variety of febrile causes—typhoid fever, pneumonia, pyelitis, empyema, influenza, and encephalitis. From Table I of their article we may collect 23 such analyses made during the febrile stage, and in this group the plasma volume averaged 52.6 $\pm$1.4* cc. per kilogram of body-weight; the concentration of chlorides, 93 $\pm$1.5 cc. n/10 solution per 100 cc. plasma; the total

[1] Submitted for publication March 7, 1930.

* The second figure represents the standard error of the mean.

TABLE I

Control rabbits. Effect on blood, plasma, and cell volumes, and on body-weight, of a repetition of the analytical procedure after an interval of 4 or 5 days between determinations I and II

RABBIT NUMBER	BLOOD VOLUME			BLOOD VOLUME PER KILOGRAM			PLASMA VOLUME			PLASMA VOLUME PER KILOGRAM			CELL VOLUME			CELL VOLUME PER KILOGRAM			WEIGHT			INTERVAL BETWEEN I AND II
	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	
	cc.	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*grams*	*grams*		*days*
101	122	119	−2.5	57.0	54.6	−4.2	83.5	81	−3.0	39.0	37.1	−4.9	38.5	38.0	−1.3	18.0	17.5	−2.8	2,140	2,180	+1.9	4
103	104	102	−1.9	48.4	48.3	−0.2	68	67	−1.5	31.6	31.8	+0.6	36	35.0	−2.8	16.8	16.5	−1.8	2,150	2,110	−1.9	5
116	88	84	−4.5	47.1	45.4	−3.6	61	57.5	−5.7	32.6	31.1	−4.6	27	26.5	−1.9	14.5	14.3	−1.4	1,870	1,850	−1.1	5
117	112	112	0.0	49.3	46.9	−4.9	80.5	77	−4.4	35.5	32.2	−9.3	31.5	35	+11.1	13.8	14.7	+6.5	2,270	2,390	+5.3	5
Mean percentage change			−2.2			−3.2			−3.7			−4.6			+1.3			+0.1			+1.1	
Standard error			0.8			0.9			0.8			1.8			2.9			2.0			1.4	
Ratio $\frac{M}{\epsilon}$			2.8			3.4			4.6			2.6			0.4			0.05			0.8	

circulating plasma chloride, calculated by multiplying the plasma volume by the plasma chloride concentration, 48.6 ±1.4 cc. n/10 solution per kilogram of body-weight. The analytical methods which they used gave in healthy persons results of 50 ±5 cc. of plasma per kilogram and 95.5 ±2.0 cc. n/10 chloride per 100 cc. of plasma, so that normal individuals might be expected to show a circulating plasma chloride content close to 48 cc. n/10 solution per kilogram of body-weight. In this series, then, fever did not appear to cause striking deviations from normal values. From Table 2 of their article, however, we may select for similar analysis the three patients whose plasma volume and chloride concentration were determined both during a febrile period and after recovery (cases 2, 29, and 33). Two of the three showed an increase in plasma chloride concentration during convalescence, while all of them had striking reductions in plasma volume, the average percentage reduction for the series being 28 ±5 per cent of the value obtained after recovery. All three showed also a reduction, during convalescence, of the total circulating plasma chloride, averaging 21 ±4 per cent of the value after recovery—the latter being assumed for purposes of calculation to be somewhere near the normal value for the individual. May one infer that, in the transition from the normal to the febrile state, changes occur of magnitude similar to those accompanying convalescence but of opposite sign? Verification of this thesis requires the use of experimental animals.

METHODS

Young rabbits were used for the most part; they were fed on oats and occasionally carrots and allowed unrestricted amounts of water, except when otherwise specified. Their weights ranged from 760 to 2390 grams, the majority around 2 kgm. The scales used in weighing were sensitive to a 2 gram increment with a load of 2500 grams. For the determination of the circulating plasma electrolyte, the animal was fixed on a board and a sample of blood taken from an ear vein for hematocrit and for analysis of serum chloride (4), bicarbonate (5), and total fixed base (6), the volume of the preliminary sample being accurately measured in a graduated centrifuge tube. The flow of blood was then temporarily checked and the total blood volume determined by the carbon monoxide method with an apparatus modelled after the one

originally described by Douglas (7). A second hematocrit determination was usually made at the end of the experiment as a check on the first; but in calculating the total plasma, either the first value or the average of both was taken. In obtaining the figure for the total circulating blood volume prior to the experimental interference the measured volume of blood removed for determination of serum electrolyte concentration was added to the volume as determined by the carbon monoxide method; thus, in these calculations it was assumed that after withdrawal of a small sample (usually 6 or 7 cc.) no appreciable compensatory influx of fluid from reserve sources into the blood stream would occur (8, 9, 10) during the interval of ten to twenty minutes elapsing between the beginning of the blood collection and the completion of blood volume determination.

The principal innovations in the method used for blood and plasma volume determinations were as follows: For the mask, a glass cylinder 5 cm. in diameter was used, over one end of which was stretched a rubber glove-finger with the tip cut out, and into the other end was inserted a rubber stopper with perforations for three inlets. In place of the motor-driven pump for absorption of carbon dioxide as described by Douglas, a hand-pump was used, consisting of a small rubber bulb with one-way valves permitting the air in the mask to be driven through 10 per cent KOH solution in a reservoir connected to the mask by an inverted funnel and large-bore rubber tubing. This solution thus served the double purpose of absorbing CO_2 and furnishing the elastic element necessary for tidal respiration. By this economy of air space the volume of residual air in the mask, pump, and funnel was only 350 cc. when the KOH solution stood at its average level during quiet breathing. Leakage of gas out of the rebreathing apparatus was obviated by Douglas' device of maintaining the pressure constantly less than atmospheric. Thus, when the mask was first applied, with the oxygen inlet tube closed, it constituted a closed rebreathing system with a volume external to the animal's respiratory system of about 350 cc.; an unmeasured portion of the contained air was now withdrawn by suction through the oxygen inlet tube, its place being taken by the KOH solution as it rose in the funnel as the result of atmospheric pressure on the KOH solution in the reservoir outside the funnel. CO and later oxygen were then admitted to the system at a rate which

would compensate for absorption of CO and O_2 by the blood and of CO_2 by the KOH solution and still not bring the pressure of the gases in the mask up to atmospheric, even at full expiration. At the end of a ten-minute breathing period, 3.5 or 4 cc. of blood were removed for carbon monoxide analysis by allowing the drops to fall from the rabbit's ear through paraffin oil into the bottom of a sealed-off funnel containing powdered sodium oxalate. A 50 cc. sample of air was then drawn from the mask for analysis of unabsorbed carbon monoxide, and the rabbit liberated.

Blood carbon monoxide content was determined by a modification of the method of Van Slyke and Robscheit-Robbins (11) previously described (12). Analysis of the residual air for carbon monoxide has also been described (12), but in the present series of experiments the method was slightly elaborated so as to include the determination of carbon dioxide, oxygen, and carbon monoxide in a single 50 cc. sample by using the Harrington-Van Slyke pipet with a gas volume of 50 cc. for manometric measurement of the first two components. It was found that the average oxygen concentration within the breathing apparatus varied considerably in different determinations, owing apparently to differences in the amounts of atmospheric nitrogen included when the mask was first applied to the rabbit and also to different rates and degrees of absorption of carbon dioxide from the rebreathed air; therefore, the amounts of carbon monoxide remaining unabsorbed by the rabbit's blood varied likewise and required separate determination by analysis for each experiment instead of correction by deduction of an average quantity such as was done by Douglas (7) and by Boycott and Douglas (9). This involved multiplying the determined concentration of carbon monoxide in the residual air by the residual air volume, part of which was measured, part of which—the dead space within the animal—could only be estimated. The first few hematocrit determinations were made according to the method of Van Allen (13); the method of Went and Drinker (14) was found more satisfactory, however, and was resorted to for all subsequent determinations. In this, we assume that the osmotic pressure exerted by the dissolved heparin was negligible. The tubes were spun rapidly for 45 minutes, preliminary tests having shown that with the centrifuge speed used no further packing of cells occurred after 30 minutes. Duplicate determinations checked usually to less than 0.6 volume per cent.

The analytical errors are about as follows: For the determination of blood volume the accuracy is probably close to 5 per cent. The use of small animals like rabbits introduces a larger error in the measurement of the small samples of gas injected into the breathing system, in comparison to the amounts required in making blood volume determinations on humans; but this is to a large extent offset by the greater accuracy that can be obtained by administering enough carbon monoxide to give a final concentration of 5 to 7 per cent in the blood, and by keeping the gases in the breathing system under a pressure constantly less than atmospheric, as referred to above. However desirable it would have been to perform blood carbon monoxide analyses in duplicate, this was not attempted because of the disturbing element introduced by the withdrawal of larger amounts of blood. Moreover, it is unlikely that the error of gas analysis is the largest factor in the combined error of the method as a whole. Total fixed base analyses are done with an error seldom greater than 1.5 to 2 per cent; chlorides, about 1 per cent; bicarbonate determinations were done in duplicate within a variation of 3 per cent between the two values, or 1.5 per cent on either side of the mean. The error of total plasma electrolyte content, involving the product of two factors each with its own error, is probably in the neighborhood of 8 per cent.

It has been assumed that the calculation of total circulating electrolyte may be made without introducing a significant error by multiplying the serum concentration of electrolyte by the figure obtained for total circulating plasma. Bicarbonate determinations were made after equilibrating the serum with alveolar air, and the total amount of carbon dioxide eliminated was calculated as bicarbonate ions; the error so introduced by failure to determine pH and to deduct the corresponding proportion of total carbon dioxide present as carbonic acid is negligible in these tabulations. The last figure in the values given for undetermined acid per 100 cc. of serum and in the total plasma is without significance, being obtained by subtracting from the total base figure the sum of chloride and bicarbonate determined.

From the observed blood volume, hematocrit, serum concentration of total fixed base, chloride, and carbon dioxide combining power, and from observed body-weight, we have calculated plasma and cell volume; blood, plasma, and cell volumes per kilogram of body-weight; total

plasma content of total fixed base, chloride, bicarbonate, and undetermined anions; and total plasma content of these four electrolyte components per kilogram of body-weight.

EXPERIMENTAL

1. Controls

Before applying procedures to the rabbits the effect of which on the total circulating plasma electrolytes was to be investigated, it was necessary to find out how much was the effect, and in what direction, of the analytical procedure itself. Boycott and Douglas (9) and Salvesen (15) report an increase of total blood volume, usually with most of the increase occurring in the plasma, in determinations subsequent to the initial one. Since the procedure used here called for the removal of 10 or 12 cc. of blood at each determination, or about 8 to 20 per cent of the total blood volume in the animals studied, it was to be expected that at the end of a period of five days or so after the first determination (I) the second determination (II) might show some readjustment of blood and plasma volume. These changes are reported in Table I, both the determined values and the percentage deviations from the initial value $\left(\frac{\text{II} - \text{I}}{\text{I}} \times 100\right)$ being given. The arithmetical mean of the percentage deviations has been calculated, the prefixing of a plus or minus sign denoting an average increase or decrease from the original figure. The standard error of the mean gives an indication of the compactness of grouping of the individual values about the average, and the ratio of mean to standard error affords a numerical gauge of the consistency of the change. In a series of four members, for example, if the value of this ratio is greater than 3, the chances are 24 to 1 or better that an individual chosen at random from a "normal" population (in the statistical sense) composed of similar individuals—*i.e.*, under similar experimental conditions—would fall within the limits of zero and twice the mean for the series. When there are but three members in a series, as in the case of Tables VIII, IX, XII, and XIII the ratio must have a value of about 3.8 in order to indicate similar odds of 24 to 1. These criteria apply to the tables throughout.

The effect of the procedure, then, as shown in Table I, is to bring

about a slight diminution of circulating blood volume—a diminution which is somewhat greater when calculated as blood volume per kilogram of body-weight, on account of the concurrent average gain in weight of the animals in the interval between the two determinations—and a diminution of plasma volume. The magnitude of this change, however, is close to the limit of error of the analytical methods used; it is probably safer on this evidence merely to conclude that no compensatory increase of blood or plasma volume has been shown to take place. Cell volume remained essentially unchanged, both absolutely and with relation to body-weight, and it may be presumed that to compensate for loss of blood of this magnitude the organism is able within the interval of four or five days elapsing between determinations to mobilize cells from stores or through new blood-cell formation so as essentially to replace the quantity lost; were this not the case, and were the loss of cells to remain uncompensated, the method of blood volume determination is sufficiently accurate to record such absence of compensation.

These results, then, do not confirm those of Boycott and Douglas quoted above as having demonstrated an increase in blood volume (chiefly in the plasma component) as the result of loss of blood; but this difference is easily attributable to the larger bleedings to which their animals were subjected (36 to 45 per cent of the original blood volume). Salvesen reported both decreases and increases in duplicate determinations made after intervals of 4 to 15 days (Tables III and IV, *loc. cit.*); but since the average in the six cases was $+4.5 \pm 5.5$ cc. change, it is not permissible to draw conclusions from his series concerning either the direction or magnitude of change.

The effect of the analytical procedure on the concentration of electrolytes in the serum and on their total amount in the circulating plasma is given in Table II. Inspection of the ratios of mean percentage change to standard error of the mean shows that significant changes occurred in the concentration of total fixed base, bicarbonate, and undetermined acids and in the total amount of circulating fixed base, chloride, and undetermined acids. All showed a diminution except the percentage concentration of bicarbonate, which was raised an average of 44 per cent. The cause of such change is not clear. These results are expressed graphically in Figure 1.

TABLE II

Control rabbits. Percentage changes in total fixed base, chloride, bicarbonate, and undetermined acids per 100 cc. of serum and in total plasma on repetition of the analytical procedure after an interval of 4 or 5 days

The figures in the I and II columns represent cubic centimeters n/10 solution

RABBIT NUMBER	TOTAL FIXED BASE PER 100 CC. SERUM			CHLORIDE PER 100 CC. SERUM			BICARBONATE PER 100 CC. SERUM			UNDETERMINED ACIDS PER 100 CC. SERUM			TOTAL FIXED BASE IN TOTAL PLASMA			CHLORIDE IN TOTAL PLASMA			BICARBONATE IN TOTAL PLASMA			UNDETERMINED ACIDS IN TOTAL PLASMA		
	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change
101	177	167	−5.7	111	111	0.0	15.5	21.0	+36	50.5	35.0	−31	148	135	−8.8	92.7	89.9	−3.0	12.9	17.0	+32	42.2	28.4	−33
103	178	160	−10.1	116	107	−7.8	14.9	27.6	+85	47.1	25.4	−46	121	107	−11.6	78.8	71.6	−9.1	10.1	18.5	+83	32.0	17.0	−47
116	167	162	−3.0	108	105	−1.8	16.8	25.1	+49	42.2	31.9	−24	102	93	−8.8	65.9	60.4	−8.3	10.2	14.4	+41	25.7	18.4	−28
117	174	164	−5.7	105	102	−2.9	21.2	22.4	+6	47.8	39.6	−17	140	126	−10.0	84.5	78.6	−7.0	17.1	17.2	+1	38.5	30.5	−21
Mean percentage change			−6.1			−3.1			+44			−30			−9.8			−6.9			+39			−32
Standard error			1.2			1.5			14			5			0.6			1.2			15			5
Ratio $\frac{M}{\epsilon}$			5.1			2.1			3.1			6.0			16.3			5.8			2.6			6.4

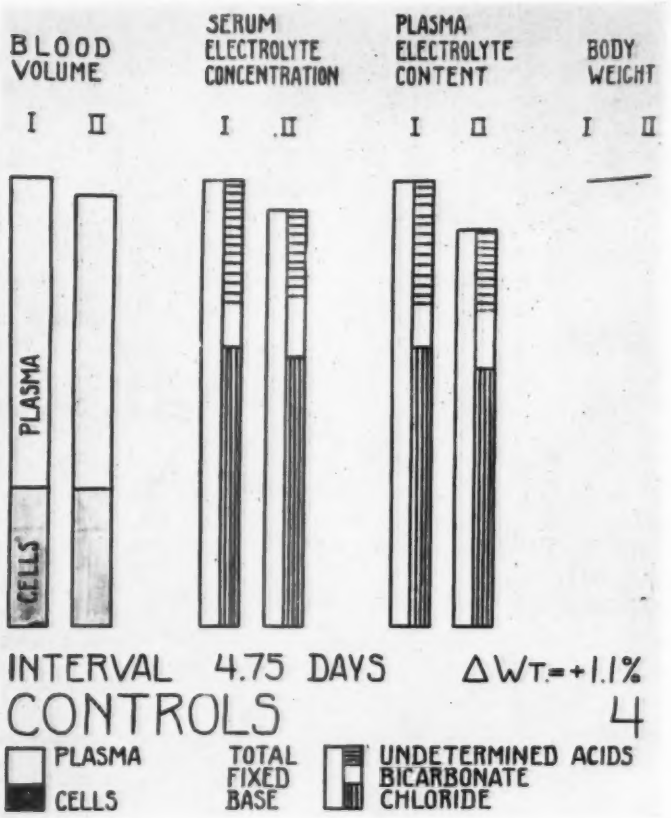

FIG. 1. CHANGES IN BLOOD VOLUME, SERUM ELECTROLYTE CONCENTRATION, TOTAL CIRCULATING PLASMA ELECTROLYTES AND BODY-WEIGHT IN THE CONTROL ANIMALS

The scales are so chosen that the tops of the columns and the starting point of the weight curve, representing the conditions at the time of the first determination (I), are on a level.

The blood volume columns and plasma electrolyte content columns represent the average of these values per kilogram of body weight.

2. Dysentery (Shiga) vaccine intraperitoneally

In the course of other experiments in this laboratory it had been found that significant changes in the serum concentration of electrolytes could often be brought about by the intraperitoneal injection of Shiga vaccine. In our first experiments on total electrolyte changes, therefore, we used this technique. The vaccine was prepared by growing a virulent strain of Shiga organisms on semi-solid media for 24 hours, washing off with normal salt solution (about 5 cc. per slant), and killing the organisms by heating at 60°C. for one hour. No determination was made of the number of organisms per cubic millimeter of the vaccine or of the amount of soluble toxin present. There was considerable lack of uniformity in the response of individual animals to injections of this preparation: one rabbit showed an apparently maximal sublethal response to 1 cc., becoming very limp and inactive, with rapid respirations, at the end of 70 minutes; others responded scarcely at all to the injection of 20 cc. With intermediate doses several rabbits were killed in from a little over one hour to something less than 24 hours, and their downward course from the moment when they commenced to show adverse symptoms was so rapid that the opportunity for making satisfactory observations of electrolyte distribution was lost. Of the four animals on which determinations were made, recorded in Tables III and IV, the dosage of vaccine was 1, 2, 10, and 10 cc. respectively, and the interval from the injection of the vaccine to the plasma electrolyte analysis 1 hour 25 minutes, 6 hours 15 minutes, 2 hours 10 minutes, and 5 hours 10 minutes, respectively. Rectal temperatures were recorded in the case of the last three animals: in rabbit No. 117 it rose from 38.6 to 40.2°C.; in 124, it fell from 39.2 to less than 37.0°C.; and in No. 125, it rose from 39.7 to 40.5°C. four hours after injection, falling slightly to 40.3°C. at the time blood was taken for analysis. All animals on which such observations were made showed a drop in temperature during the determination of blood volume. This was rather unexpected, in view of the high vapor tension in the atmosphere breathed by the animal during this part of the test and the dependence of the rabbit on pulmonary ventilation for temperature regulation.

Tables III and IV show that there is, in general, in response to the

TABLE III

Shiga bacillus vaccine given intraperitoneally a few hours before determination II

RABBIT NUMBER	BLOOD VOLUME			BLOOD VOLUME PER KILOGRAM			PLASMA VOLUME			PLASMA VOLUME PER KILOGRAM			CELL VOLUME			CELL VOLUME PER KILOGRAM			WEIGHT			INTERVAL BETWEEN I AND II
	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	
	cc.	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*grams*	*grams*		*days*
116	84	106	+26.2	45.4	59.5	+31.0	57.5	74.0	+28.7	31.1	41.6	+33.8	26.5	32	+20.8	14.3	17.9	+25.2	1,850	1,780	−3.8	4
117	112	110	−1.8	46.9	47.2	+0.6	77	79	+2.6	32.2	33.9	+5.3	35	31	−11.4	14.7	13.3	−9.5	2,390	2,330	−2.5	5
124	63.5	69	+8.7	52.7	60.1	+14.0	41.5	45	+8.4	34.5	39.2	+13.6	22	24	+9.1	18.2	20.9	+14.8	1,205	1,148	−4.7	6
125	50.5	56.5	+11.9	66.6	66.5	−0.2	34	39.5	+16.2	44.7	46.5	+4.0	16.5	17	+3.0	21.9	20.0	−8.7	760	850	+11.8	6
Mean percentage change.....			+11.3			+11.4			+14.0			+14.2			+5.4			+5.5			+0.2	
Standard error.....			5.0			6.3			4.9			6.0			5.8			7.5			3.4	
Ratio $\frac{M}{\epsilon}$.....			2.3			1.8			2.9			2.4			0.9			0.7			0.06	

TABLE IV

Shiga bacillus vaccine given intraperitoneally a few hours before determination II

RABBIT NUMBER	TOTAL FIXED BASE PER 100 CC. SERUM			CHLORIDE PER 100 CC. SERUM			BICARBONATE PER 100 CC. SERUM			UNDETERMINED ACIDS PER 100 CC. SERUM			TOTAL FIXED BASE IN TOTAL PLASMA			CALORIDE IN TOTAL PLASMA			BICARBONATE IN TOTAL PLASMA			UNDETERMINED ACIDS IN TOTAL PLASMA		
	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change
116	162	158	−2.5	105	109	+3.8	25.1	20.6	−17.9	31.9	28.4	−11.0	93.2	116.9	+25.4	60.4	80.7	+33.6	14.4	15.3	+6.3	18.4	21.0	+14.1
117	164	165	+0.6	102	105	+2.9	22.4	22.3	0.0	39.6	37.7	−4.8	126.4	130.4	+3.2	78.6	82.9	+5.5	17.2	17.6	+2.3	30.5	29.8	−2.3
124	172	160	−7.0	103	102	−1.0	24.1	6.8	−71.8	44.9	51.2	+14.0	71.4	72.0	+0.8	42.8	45.9	+7.2	10.0	3.1	−69.0	18.6	23.0	+23.7
125	171	170	−0.6	101	104	+3.0	24.7	23.7	−4.1	45.3	42.3	−6.6	58.1	67.2	+15.7	34.3	41.1	+19.8	8.4	9.4	+11.9	15.4	16.7	+8.4
Mean percentage change			−2.4			+2.2			−24			−2.1			+11.3			+16.5			−12			+11.0
Standard error			1.4			1.0			14			4.8			5.0			5.9			17			4.7
Ratio $\frac{M}{\epsilon}$			1.7			2.2			1.7			0.4			2.3			2.8			0.7			2.3

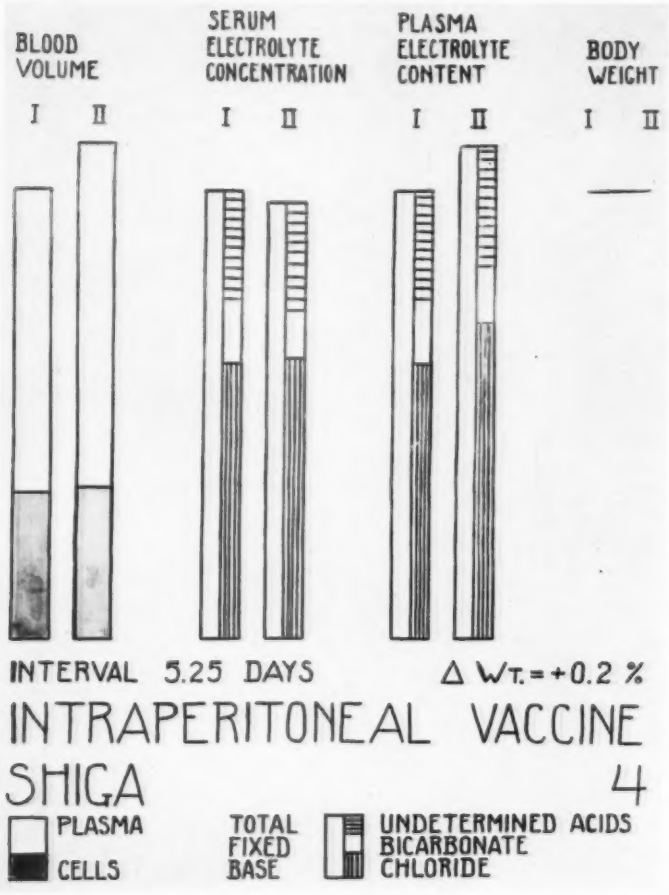

FIG. 2. CHANGES IN BLOOD VOLUME, SERUM ELECTROLYTE CONCENTRATION, TOTAL CIRCULATING PLASMA ELECTROLYTES AND BODY-WEIGHT IN THE ANIMALS INJECTED INTRAPERITONEALLY WITH SHIGA VACCINE

See legend under Figure 1

TABLE V

B. coli vaccine given intraperitoneally a few hours before determination II

Rabbit 135. Interval between I and II, 15 days

	BLOOD VOLUME	BLOOD VOLUME PER KILOGRAM	PLASMA VOLUME	PLASMA VOLUME PER KILOGRAM	CELL VOLUME	CELL VOLUME PER KILOGRAM	WEIGHT	TOTAL FIXED BASE PER 100 CC. SERUM	CHLORIDE PER 100 CC. SERUM	BICARBONATE PER 100 CC. SERUM	UNDETERMINED ACIDS PER 100 CC. SERUM	TOTAL FIXED BASE IN TOTAL PLASMA	CHLORIDE IN TOTAL PLASMA	BICARBONATE IN TOTAL PLASMA	UNDETERMINED ACIDS IN TOTAL PLASMA
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*grams*	*cc. n/10*	*cc. n/10*	*cc. n/10*	*cc. n/10*	*cc. n/10*	*cc. n/10*	*cc. n/10*	*cc. n/10*
I	109.5	52.9	67.5	32.6	42	20.3	2,070	156	97	28.0	31.0	105.3	65.5	18.9	20.9
II	115	60.5	72	37.9	43	22.6	1,900	169	103	19.4	46.6	121.7	74.2	14.0	33.5
Percentage change.....	+5.0	+14.4	+6.7	+16.3	+2.4	+11.3	−8.2	+8.3	+6.2	−30.7	+50.3	+15.6	+13.3	−25.9	+60.2

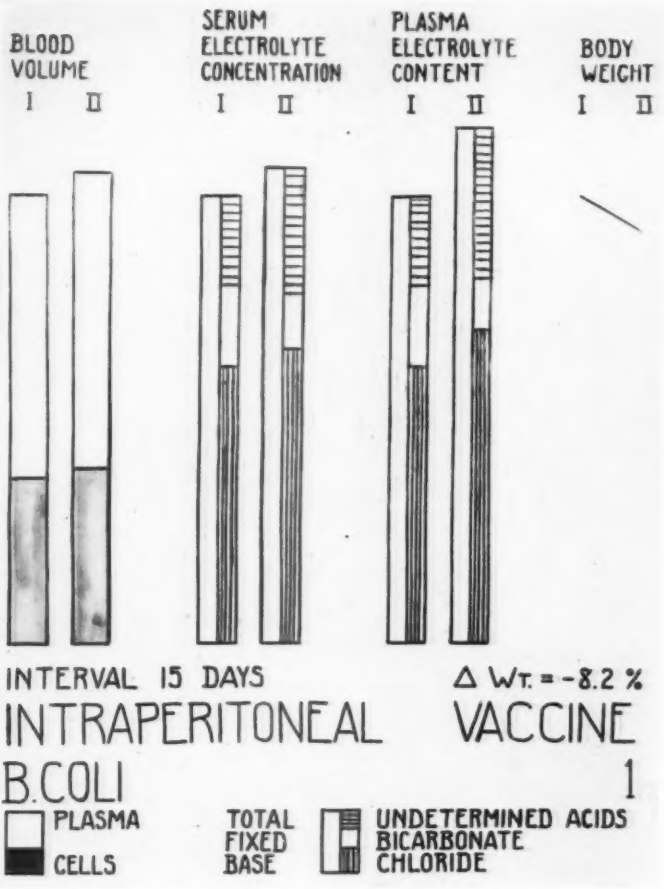

FIG. 3. CHANGES IN BLOOD VOLUME, SERUM ELECTROLYTE CONCENTRATION, TOTAL CIRCULATING PLASMA ELECTROLYTES AND BODY-WEIGHT IN THE ANIMAL INJECTED INTRAPERITONEALLY WITH B. COLI VACCINE

The blood volume columns and plasma electrolyte content columns represent absolute values, not per kilogram of body-weight.

intraperitoneal injection of Shiga vaccine, an elevation (averaging 11 per cent) of total circulating blood volume occurring chiefly in the plasma component (14 per cent) and uninfluenced by concomitant changes in body-weight, together with a diminution in serum bicarbonate concentration and an increase in the total amount of circulating base, chloride, and undetermined acids, with an inconstant change in the total circulating bicarbonate. But on the whole little more than the general trend of reaction is indicated by these determinations. They are graphically represented in Figure 2.

3. Intraperitoneal injection of B. coli vaccine

In the preceding group the Shiga bacillus had not been chosen for any specific effect of that organism, but it seemed desirable to find out whether some other organism would give a similar response. One rabbit was therefore given an intraperitoneal injection of 20 cc. of a vaccine made by a similar technique from a strain of B. coli (Table V and Fig. 3). At the end of 5 hours 20 minutes after the injection, the animal looked somewhat sick and limp and showed slight hyperpnea and a rise of temperature from 38.4 to 39.0°C. Analysis at that time showed changes from the values obtained 15 days previously, similar to those caused by the Shiga organism: an increase in total blood volume, contributed mainly by a 7 per cent rise in plasma volume; a rise of 16 per cent in total circulating fixed base, of 13 per cent in total chloride, and of 60 per cent in total undetermined acids; a fall of 26 per cent in total bicarbonate. Although the interval between the first and second determinations was long, these changes could not be ascribed to simple growth, since the body-weight during this period had fallen 8.2 per cent and the alterations in electrolyte distribution per kilogram of body-weight were even more marked than the absolute changes.

4. Intravenous injection of B. coli vaccine

The attempt was next made to overcome the irregularity of response to intraperitoneal injection of vaccine by giving it intravenously. One cubic centimeter of the vaccine described under Section 3 was administered to each of four rabbits through the posterior ear vein. Each animal was closely watched in regard to its general behavior,

and its temperature taken about once an hour; as soon as it showed marked depression of activity or increase in the rapidity of respiration or both, blood analyses were made. The interval elapsing between injection of the vaccine and electrolyte determination was 5 hours 10 minutes, 4 hours 10 minutes, 1 hour 55 minutes, and 4 hours 25 minutes, respectively. The temperature records of these animals are given in Figure 4. The changes suggested by the preliminary experiments

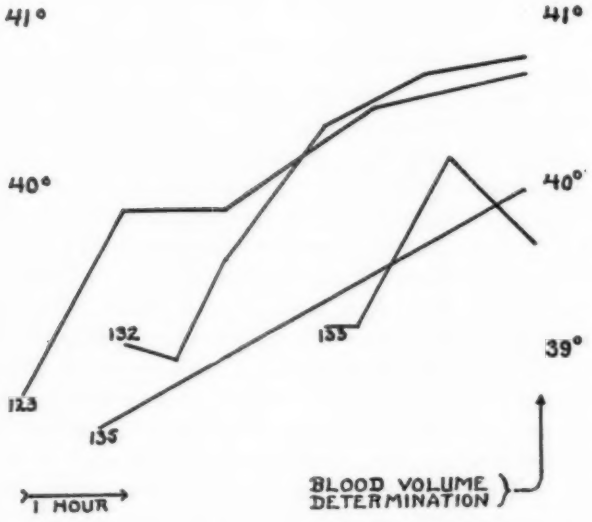

FIG. 4. RECTAL TEMPERATURE CURVES OF RABBITS INJECTED INTRAVENOUSLY WITH B. COLI VACCINE

Injection was made immediately after the beginning of each curve.

recorded above now became unequivocal and fell well outside the experimental error of the methods used. In Table VI it will be seen that there resulted an increase in total blood volume which averaged 12 per cent for the entire series of four animals; that this increase was essentially limited to the plasma component, which rose 20 per cent above its initial value; that there was no significant change in the volume of cells; and, finally, that these alterations were not simply the result

TABLE VI

B. coli vaccine given intravenously shortly before determination II

RABBIT NUMBER	BLOOD VOLUME			BLOOD VOLUME PER KILOGRAM			PLASMA VOLUME			PLASMA VOLUME PER KILOGRAM			CELL VOLUME			CELL VOLUME PER KILOGRAM			WEIGHT			INTERVAL BETWEEN I AND II
	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	
	cc.	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*grams*	*grams*		*days*
123	72	75.5	+4.9	55.5	64.1	+15.5	45	51.5	+14.5	34.7	43.8	+26.2	27	24	−11.1	20.8	20.3	−2.4	1,297	1,177	−9.3	6
132	103	110	+6.8	64.8	75.0	+15.8	64.5	77.5	+20.2	40.6	52.9	+30.3	38.5	32.5	−15.6	24.2	22.1	−8.7	1,590	1,468	−7.7	22
133	112.5	140	+24.4	65.6	86.0	+31.1	74	100	+35.1	43.2	61.5	+42.4	38.5	40	+3.9	22.4	24.5	+9.4	1,715	1,628	−5.1	8
135	100.5	112.5	+11.9	55.6	60.1	+8.1	66.5	72.5	+ 9.0	36.8	38.7	+5.2	34	40	+17.7	18.8	21.4	+13.8	1,807	1,871	+3.5	11
Mean percentage change			+12.0			+17.6			+19.7			+26.0			−1.3			+3.0			−4.7	
Standard error			3.8			4.2			4.9			6.7			6.6			4.5			2.5	
Ratio $\frac{M}{\epsilon}$			3.2			4.2			4.0			3.9			0.2			0.7			1.9	

TABLE VII

B. coli vaccine given intravenously shortly before determination II

RABBIT NUMBER	TOTAL FIXED BASE PER 100 CC. SERUM			CHLORIDE PER 100 CC. SERUM			BICARBONATE PER 100 CC. SERUM			UNDETERMINED ACIDS PER 100 CC. SERUM			TOTAL FIXED BASE IN TOTAL PLASMA			CHLORIDE IN TOTAL PLASMA			BICARBONATE IN TOTAL PLASMA			UNDETERMINED ACIDS IN TOTAL PLASMA		
	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change
123	163	164	+0.6	108	109	+0.9	23.1	18.7	−19	31.9	36.3	+14	73.4	84.5	+14.9	48.6	56.2	+15.6	10.4	9.6	−7.7	14.4	18.7	+30
132	174	161	−7.5	110	108	−1.8	22.2	19.9	−10	41.8	33.1	−21	112.2	124.8	+11.2	71.0	83.7	+17.9	14.3	15.4	+7.7	26.9	25.7	−5
133	168	174	+3.6	108	110	+1.9	23.0	8.6	−63	37.0	55.4	+50	124.3	174	+40.0	80.0	110	+37.5	17.0	8.6	−49.4	27.3	55.4	+103
135	170	172	+1.2	102	108	+5.9	23.9	21.5	−10	44.1	42.5	−4	113.1	124.7	+10.3	67.8	78.3	+15.5	15.9	15.6	−1.9	29.4	30.8	+5
Mean percentage change....			−0.5			+1.7			−26			+10			+19			+22			−13			+33
Standard error.....			2.1			1.4			11			13			6			5			11			21
Ratio $\frac{M}{\epsilon}$.....			0.2			1.2			2.4			0.8			3.2			4.4			1.2			1.6

of growth in the interval between the two determinations, since the average weight change was a loss of about 5 per cent. In spite of inconstant changes in the serum concentration of electrolytes (Table

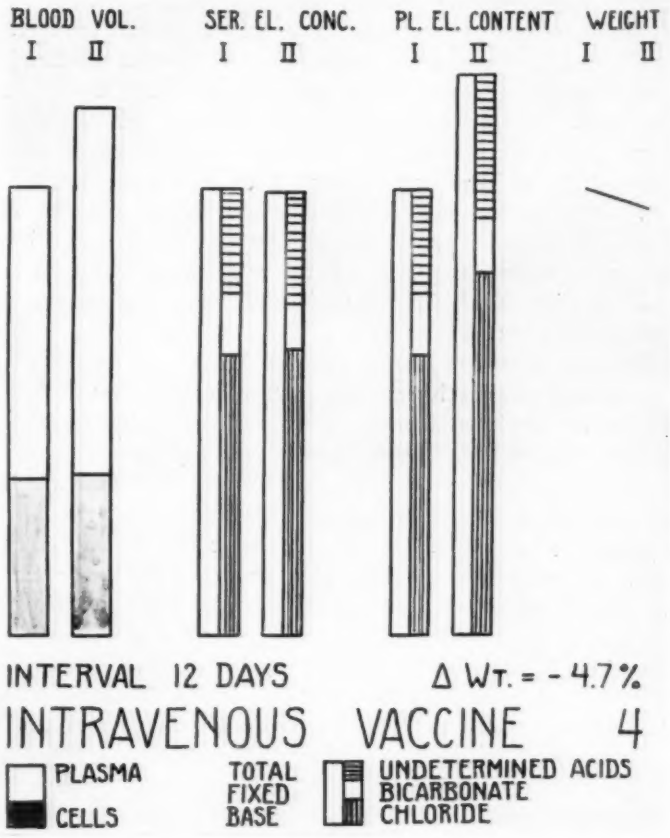

FIG. 5. CHANGES IN BLOOD VOLUME, SERUM ELECTROLYTE CONCENTRATION, TOTAL CIRCULATING PLASMA ELECTROLYTES AND BODY-WEIGHT IN THE ANIMALS INJECTED INTRAVENOUSLY WITH B. COLI VACCINE

See legend under Figure 1

VII), the total circulating fixed base in the plasma showed an increase of 19 per cent and the chloride an increase of 22 per cent. The results are shown also in Figure 5.

With the method used for influencing the electrolyte distribution of the experimental animals, namely, the intravenous injection of a vaccine, considerable variations in individual behavior were to be expected. It is of interest to examine the tables (VI and VII) with this in mind. The most marked changes in blood and plasma volume, in total fixed base, chloride, and undetermined acids in the total circulating plasma occurred in the rabbit (No. 133) which showed the most appreciable lowering of serum bicarbonate concentration as well as of total circulating plasma bicarbonate. As a result of the vaccine injection, all of the animals studied showed a certain degree of depression of serum bicarbonate concentration; but one of them (No. 132) experienced an increase in the volume of circulating plasma which was great enough to compensate for this lowering of concentration so that the net result was an increase of total circulating bicarbonate. The behavior of the undetermined acid fraction, both in regard to its changes in concentration and in total amount in circulation, were inconstant both in degree and direction. Thus, although the exact composition of this fraction was not explored in detail, it is probably permissible to infer that as a result of the vaccine injection there was no very considerable constant mobilization of any one particular acid radical, such as lactate, which might be included in this group. Since the proportion of base bound by protein increases with increase in pH, and since the latter change is not likely to occur in the presence of a falling serum bicarbonate, it is equally improbable that the procedure brought about any constant increase in the base bound by protein; but in the absence of protein determinations no definite conclusions as to the behavior of protein anions are justified by these experiments. Two of the animals (Nos. 133 and 135) showed a considerable mobilization of cells in response to vaccine injection. This is consistent with the evidence that Barcroft (16) has adduced regarding the function of the spleen. On the other hand, changes in cells in the direction of reduction of volume also occurred twice in this group; but without other evidence we are not prepared to ascribe this to any specific factor such as destruction or segregation. A proper interpretation of cell volume changes doubtless hinges on further study in this field.

5. The recovery process

While it is unfortunate for a study of this kind that the nature of the experimental methods used precluded repeated observations of the animals' plasma electrolyte content at frequent intervals, it nevertheless seemed desirable to find out, even if only in a rough way, whether these changes as given in Tables VI and VII were lasting or merely transitory. It was also felt that if a return of plasma and electrolyte content to the original base levels could be satisfactorily demonstrated this would constitute to a considerable extent a confirmation of the reliability of the analytical procedure. The results of such determinations in three animals after a recovery period of 13, 8, and 6 days, respectively, are given in Tables VIII and IX, in which the figures under the II columns are the same as those of the II columns in Tables VI and VII above and represent the levels found at the height, or somewhere near the height, of the vaccine effect. The percentage changes are figured on the basis of the deviations from the starting levels of the experiments on these rabbits, as given in Tables VI and VII, $\left(\text{Per cent change} = \frac{100\ (\text{III} - \text{II})}{\text{I}}\right)$, in order to permit a comparison of the alterations caused by the vaccine injection with those experienced in the recovery period.

There is evident a strong tendency toward return of blood volume and blood volume per kilogram to the original level; it is effected, however, only in part by a reduction of the plasma volume from the high level reached after vaccine injection, partly also by a reduction both absolute and relative to body-weight in the cell volume. This phenomenon may have some bearing on the part which repeated infections have in causing both relative and actual anemia. The behavior of the body-weight of the animals during the course of these experiments, showing an average fall after vaccine injection and a consistent rise in the recovery period, suggests that the changes in blood and plasma volume demonstrated are not brought about by a rise in the total amount of body fluid following the injection of vaccine, but rather are due to a redistribution of body fluid between circulatory and extracirculatory depots in favor of the blood stream, even at a time when the total fluid content of the body is either stationary or decreasing. There occurs in the recovery period also a consistent and quite appre-

TABLE VIII

Recovery after intravenous injection of B. coli vaccine

The vaccine was given shortly before determination II. Percentage changes, $\frac{100\ (III - II)}{I}$, are figured on the basis of deviations from the control values of determination I (Table VI).

RABBIT NUMBER	BLOOD VOLUME			BLOOD VOLUME PER KILOGRAM			PLASMA VOLUME			PLASMA VOLUME PER KILOGRAM			CELL VOLUME			CELL VOLUME PER KILOGRAM			WEIGHT			INTERVAL BETWEEN II AND III
	II	III	Per cent change	II	III	Per cent change	II	III	Per cent change	II	III	Per cent change	II	III	Per cent change	II	III	Per cent change	II	III	Per cent change	
	cc.	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*grams*	*grams*		*days*
123	75.5	64.5	−15.3	64.1	50.4	−24.7	51.5	45	−14.4	43.8	35.1	−25.1	24	19.5	−16.7	20.3	15.3	−24.0	1,177	1,281	+8.0	13
132	110	90.5	−18.9	75.0	55.5	−30.1	77.5	59.5	−27.9	52.9	36.5	−40.4	32.5	31	−3.9	22.1	19.0	−12.8	1,468	1,630	+10.2	8
135	112.5	107.5	−5.0	60.1	56.6	−6.3	72.5	73	+0.8	38.7	38.4	−0.8	40	34.5	−13.8	21.4	18.2	−17.0	1,871	1,900	+1.6	6
Mean percentage change			−13.1			−20.4			−13.8			−22.1			−11.5			−18			+6.6	
Standard error			3.4			5.9			6.8			9.4			3.2			3			2.1	
Ratio $\frac{M}{\epsilon}$			3.9			3.5			2.0			2.4			3.6			6			3.1	

TABLE IX

Recovery after intravenous injection of B. coli vaccine

The vaccine was given shortly before determination II. Percentage changes, $\frac{100\,(\text{III} - \text{II})}{\text{I}}$, are figured on the basis of deviations from the control values of determination I (Table VII).

Rabbit number	Total fixed base per 100 cc. serum			Chloride per 100 cc. serum			Bicarbonate per 100 cc. serum			Undetermined acids per 100 cc. serum			Total fixed base in total plasma			Chloride in total plasma			Bicarbonate in total plasma			Undetermined acids in total plasma		
	II	III	Per cent change	II	III	Per cent change	II	III	Per cent change	II	III	Per cent change	II	III	Per cent change	II	III	Per cent change	II	III	Per cent change	II	III	Per cent change
123	164	169	+3.1	109	101	−7.4	18.7	28.3	+42	36.3	39.7	+10.7	84.5	76.1	−11.4	56.2	45.5	−22.0	9.6	12.7	+30	18.7	17.9	−5.6
132	161	167	+3.4	108	103	−4.5	19.9	28.0	+27	33.1	36.0	+6.9	124.8	99.4	−22.6	83.7	61.3	−31.5	15.4	16.7	+9	25.7	21.4	−16.0
135	172	168	−2.4	108	105	−2.9	21.5	23.4	+8	42.5	39.6	−6.6	124.7	122.6	−1.9	78.3	76.7	−2.4	15.6	17.1	+9	30.8	28.8	−6.8
Mean percentage change			+1.4			−4.9			+29			+3.7			−12.0			−18.6			+16			−9.5
Standard error			1.5			1.1			9			4.3			4.9			7.0			6			2.7
Ratio $\frac{M}{\epsilon}$			0.9			4.5			3.2			0.9			2.5			2.7			2.7			3.5

ciable drop in the concentration of serum chloride and an increase of bicarbonate. The changes in the total plasma content of electrolytes are consistently opposite in direction to those brought about by vaccine injection, although there is not much uniformity in the degree of change; a much larger series of experiments would ostensibly be required in order to smooth out the differences of individual behavior.

6. Second injections of vaccine

Two of the animals already studied after a first intravenous injection of B. coli vaccine were subjected to a second similar injection. They responded with a rise in temperature to 40.6 and 40.2°C., respectively—somewhat less than they had shown after the first experiment. In Tables X and XI, in which the results of these determinations are given, the figures in the I columns correspond to those in the III columns in Tables VIII and IX representing the end of the recovery period following the first injection. Here the changes are particularly difficult to evaluate, since one of the animals gained such a large amount of weight (14 per cent) in the fourteen-day period intervening between the first and second determinations that any increase in blood volume might well have been due to this factor alone; and, in fact, while its plasma volume increased only 1 cc. absolutely, it fell appreciably in its relation to body-weight. The other rabbit, which did not gain so much weight during the period between determinations, showed changes in blood and plasma volume similar to those brought about by the first injection, but of lesser degree. As regards the electrolyte distribution, both animals showed a well-defined elevation of chloride with a fall in both bicarbonate and undetermined acids.

Thus, keeping in mind the reservations in interpretation of evidence required by the small series and in some respects the lack of uniform response in this experiment, one may take these results as suggesting (1) that after a second intravenous injection of vaccine the animals tend to respond with less marked changes in blood and plasma volume than were effected by the first injection, and (2) that a second injection, while it may or may not cause an increase in the total electrolyte content of the circulating plasma, may bring about an increase in both the concentration and total content of plasma chloride at the expense of the bicarbonate and undetermined acid fractions.

TABLE X

Response to a second intravenous injection of B. coli vaccine, given shortly before determination II

RABBIT NUMBER	BLOOD VOLUME			BLOOD VOLUME PER KILOGRAM			PLASMA VOLUME			PLASMA VOLUME PER KILOGRAM			CELL VOLUME			CELL VOLUME PER KILOGRAM			WEIGHT			INTERVAL BETWEEN I AND II	INTERVAL BETWEEN FIRST AND SECOND INJECTIONS OF VACCINE
	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change		
	cc.	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*grams*	*grams*		*days*	*days*
123	64.5	71.5	+10.8	50.4	48.9	−3.0	45	46	+2.2	35.1	31.5	−10.3	19.5	25.5	+30.8	15.3	17.4	+13.7	1,281	1,462	+14.1	14	27
132	90.5	95	+5.0	55.5	57.3	+3.2	59.5	63	+5.9	36.5	38.0	+3.9	31	32	+3.2	19.0	19.3	+1.6	1,630	1,657	+1.7	7	15
Mean percentage change.....			+7.9			+0.1			+4.1			−3.2			+17			+7.7			+8		

TABLE XI

Response to a second intravenous injection of B. coli vaccine, given shortly before determination II

RABBIT NUMBER	TOTAL FIXED BASE PER 100 CC. SERUM			CHLORIDE PER 100 CC. SERUM			BICARBONATE PER 100 CC. SERUM			UNDETERMINED ACIDS PER 100 CC. SERUM			TOTAL FIXED BASE IN TOTAL PLASMA			CHLORIDE IN TOTAL PLASMA			BICARBONATE IN TOTAL PLASMA			UNDETERMINED ACIDS IN TOTAL PLASMA		
	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change
123	169	160	−5.3	101	112	+10.9	28.3	18.4	−35	39.7	29.6	−25	76.1	73.6	−3.3	45.5	51.5	+13.2	12.7	8.5	−33	17.9	13.6	−24
132	167	168	+0.6	103	118	+14.6	28.0	21.8	−22	36.0	28.2	−22	99.4	105.8	+6.4	61.3	74.3	+21.2	16.7	13.7	−18	21.4	17.8	−17
Mean percentage change....			−2.3			+12.8			−29			−24			+1.6			+17.2			−26			−21

All of the animals in the preceding experiments were allowed free access to the drinking water supply up to the time they were placed on the board for blood determinations.

7. Effect of radiant heat

It now became of interest to determine whether the alterations in blood and plasma volume and in electrolyte distribution, as described above, could be brought about as the result of elevation of body temperature alone without the use of vaccine injection. To effect this, we subjected three rabbits to irradiation with light waves of long wave-length by

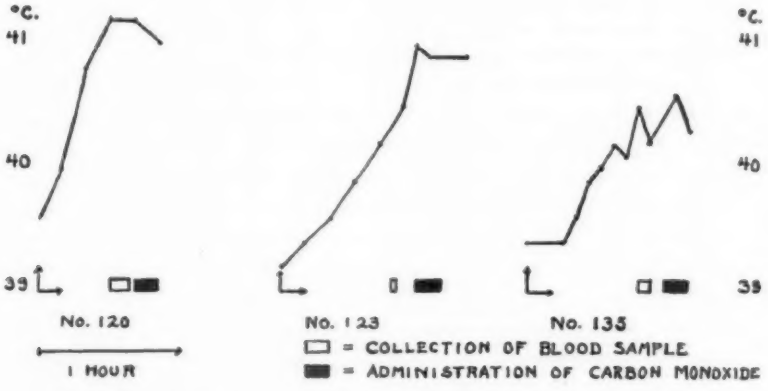

FIG. 6. RECTAL TEMPERATURE CURVES OF THE IRRADIATED RABBITS

exposing them at a distance of about one meter to a Thermolite lamp. The animals were placed on the board and exposed to the field of radiations over periods of 30 to 45 minutes, the skin being constantly massaged to promote circulation and diminish the risk of a local burn. Rectal temperatures were taken every 10 minutes or oftener, and when a well-marked fever had been produced the sample of blood was taken for serum electrolyte concentration and immediately afterward the blood volume determined. The temperature curves of the three rabbits so studied are reproduced in Figure 6. Rabbit No. 135 died as a result of the experimental procedure, and the final blood sample for

TABLE XII

Response to radiant heat applied before and during determination II

RABBIT NUMBER	BLOOD VOLUME			BLOOD VOLUME PER KILOGRAM			PLASMA VOLUME			PLASMA VOLUME PER KILOGRAM			CELL VOLUME			CELL VOLUME PER KILOGRAM			WEIGHT			INTERVAL BETWEEN I AND II
	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	
	cc.	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		*grams*	*grams*		*days*
135	88	94	+6.8	55.6	59.4	+6.8	54.5	64	+17.4	34.5	40.4	+17.1	33.5	30	−10.5	21.1	19.0	−10.0	1,582	1,583	+0.1	4
120	73	91	+24.7	68.3	79.1	+15.8	52	69.5	+33.7	48.6	60.8	+25.1	21	21.5	+2.4	19.7	18.3	−7.2	1,069	1,144	+7.0	7
123	65.5	70.5	+7.6	44.5	48.8	+9.7	44.5	48	+7.9	30.2	33.2	+9.9	21	22.5	+7.1	14.3	15.6	+9.1	1,471	1.445	−1.8	5
Mean percentage change			+13.0			+10.8			+19.7			+17.4			−0.3			−2.7			+1.8	
Standard error			4.8			2.2			6.1			3.6			4.3			4.9			2.2	
Ratio $\frac{M}{\epsilon}$			2.7			4.9			3.2			4.8			0.07			0.6			0.8	

carbon monoxide concentration used in determining the blood volume was obtained by post-mortem cardiac puncture. No water was given

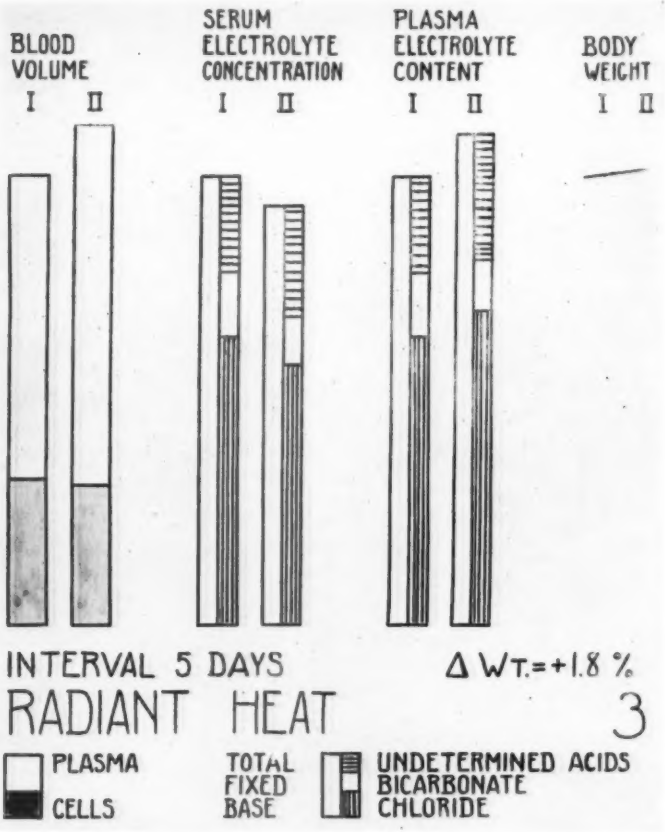

FIG. 7. CHANGES IN BLOOD VOLUME, SERUM ELECTROLYTE CONCENTRATION, TOTAL CIRCULATING PLASMA ELECTROLYTES AND BODY-WEIGHT IN THE IRRADIATED ANIMALS

See legend under Figure 1

to any of the animals from the time of commencement of the radiation until the end of the blood volume determination. The weight figures

TABLE XIII

Response to radiant heat applied before and during determination II

RABBIT NUMBER	TOTAL FIXED BASE PER 100 CC. SERUM			CHLORIDE PER 100 CC. SERUM			BICARBONATE PER 100 CC. SERUM			UNDETERMINED ACIDS PER 100 CC. SERUM			TOTAL FIXED BASE IN TOTAL PLASMA			CHLORIDE IN TOTAL PLASMA			BICARBONATE IN TOTAL PLASMA			UNDETERMINED ACIDS IN TOTAL PLASMA		
	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change	I	II	Per cent change
135	173	163	−5.8	111	102	−8.1	22.6	19.8	−12	39.4	41.2	+4.6	94.3	104.3	+10.6	60.5	65.3	+7.9	12.3	12.7	+3	21.5	26.3	+22
120	149	133	−10.7	97	83	−14.4	21.2	8.5	−60	30.8	41.5	+34.7	77.5	92.4	+19.2	50.4	57.7	+14.5	11.0	5.9	−46	16.1	28.8	+79
123	162	156	−3.7	105	107	+1.9	25.0	21.4	−14	32.0	27.6	−13.8	72.1	74.9	+3.9	46.7	51.4	+10.1	11.1	10.3	−7	14.3	13.2	−8
Mean percentage change...			−6.7			−6.9			−29			+9			+11.2			+10.8			−17			+31
Standard error.....			1.7			3.9			13			12			3.6			1.6			12			21
Ratio $\frac{M}{\epsilon}$.....			3.9			1.8			2.2			0.8			3.1			6.8			1.4			1.5

used in calculating the ratios of blood, plasma, and cell volumes to body-weight were those obtained at the end of the determinations and before any water had been given.

It will be seen from Table XII and Figure 7 that all of the animals showed a significant elevation of blood volume as a result of the experimental procedure, and that this elevation was roughly proportional to the degree of elevation of body temperature above an arbitrary normal value of 39°C. (though not proportional to the elevation above the starting level in the individual cases). The average increase so produced was well outside the limit of error of the determination. In proportion to body-weight the elevation was less marked but more consistent in degree. The change so effected occurred in the plasma fraction, no consistent change being observable in the cell fraction. The average weight change was inconsiderable, measured over the interval between the two blood volume determinations—not the interval between the commencement and the termination of heat radiation. Table XIII shows that there was a consistent fall in total fixed base concentration in the plasma, amounting to about 7 per cent of the original figure. There was also a consistent drop in plasma bicarbonate concentration, although in degree it varied in the individual animals from 12 to 60 per cent. The total plasma content of fixed base showed a consistent rise, averaging about 11 per cent. In other words, the fall in concentration of fixed base in the plasma was more than compensated by the increase in plasma volume. A similar rise in plasma chloride content, averaging 11 per cent, occurred. It will be seen on comparison of Table XIII with Table VII that these changes were quite similar to those brought about by the intravenous injection of vaccine.

8. Subcutaneous administration of normal salt solution after intraperitoneal injection of Shiga vaccine

Only one animal was included in this study, but the changes which it showed were definite. Thirteen days after the preliminary determination, the rabbit was given an intraperitoneal injection of 20 cc. of Shiga vaccine. Beginning one-half hour after this injection, it was given normal salt solution subcutaneously in 20 cc. doses approximately every hour until it had had four doses and the temperature had risen from 39.0 to 40.2°C. Forty-five minutes after the last subcu-

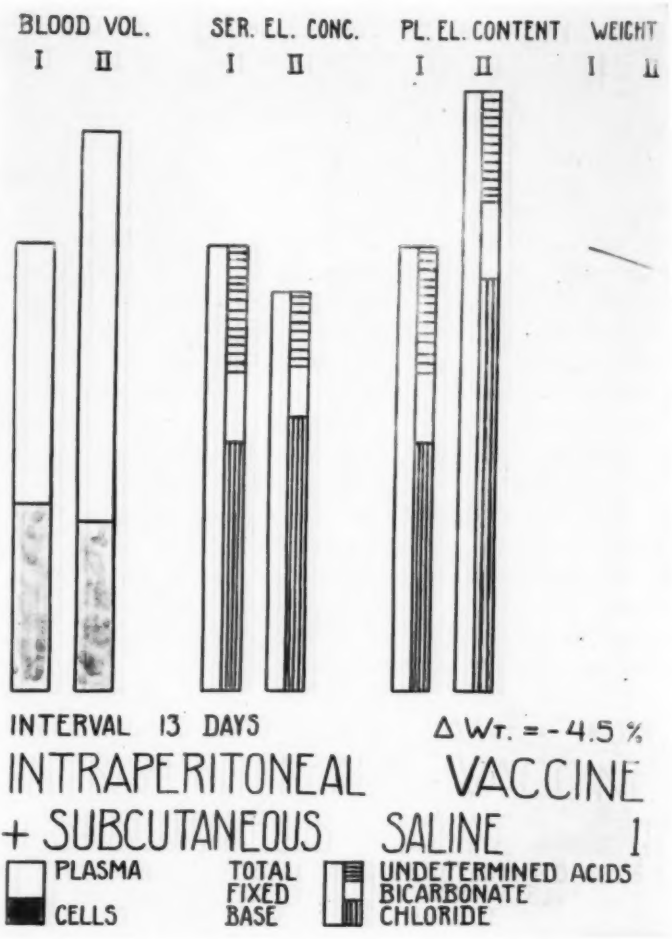

FIG. 8. CHANGES IN BLOOD VOLUME, SERUM ELECTROLYTE CONCENTRATION TOTAL CIRCULATING PLASMA ELECTROLYTES AND BODY-WEIGHT IN THE ANIMAL GIVEN NORMAL SALT SOLUTION SUBCUTANEOUSLY AFTER AN INTRAPERITONEAL INJECTION OF SHIGA VACCINE

See legend under Figure 3

TABLE XIV

Effect of intraperitoneal injection of Shiga vaccine, followed by subcutaneous injection of normal salt solution, a short time before determination II

Rabbit 134. Interval between I and II, 13 days

	BLOOD VOLUME	BLOOD VOLUME PER KILOGRAM	PLASMA VOLUME	PLASMA VOLUME PER KILOGRAM	CELL VOLUME	CELL VOLUME PER KILOGRAM	WEIGHT	TOTAL FIXED BASE PER 100 CC. SERUM	CHLORIDE PER 100 CC. SERUM	BICARBONATE PER 100 CC. SERUM	UNDETERMINED ACIDS PER 100 CC. SERUM	TOTAL FIXED BASE IN TOTAL PLASMA	CHLORIDE IN TOTAL PLASMA	BICARBONATE IN TOTAL PLASMA	UNDETERMINED ACIDS IN TOTAL PLASMA
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*grams*	*cc. n/10*	*cc. n/10*	*cc. n/10*	*cc. n/10*	*cc. n/10*	*cc. n/10*	*cc. n/10*	*cc. n/10*
I	111.5	50.2	64.5	29.1	47	21.2	2,220	178	100	27.4	50.6	114.8	64.5	17.7	32.6
II	139.5	65.8	97	45.8	42.5	20.0	2,120	160	110	20.3	29.7	155.2	106.7	19.7	28.8
Percentage change.....	+25.1	+31.1	+35	+57	−9.6	−5.7	−4.5	−10.1	+10.0	−26	−41	+35.2	+65.4	+11.3	−11.7

taneous injection, the plasma electrolyte content was determined. The results are presented in Table XIV and Figure 8. They show that when the tissue fluid store is thus augmented by subcutaneous injection of normal salt solution the tendency for the plasma requirement of extra fluid and electrolyte to be met by a redistribution of these factors is exaggerated, and that a lowering of the serum concentration of total fixed base and bicarbonate may be offset by a migration of electrolyte-containing fluid into the plasma so that the net result is a gain in plasma electrolyte. Such functions of the plasma as osmotic pressure and hydrogen-ion concentration are, of course, determined by considerations of concentration of its components in unit volume, and not by their total content in the plasma; therefore, in the determination of these functions the total plasma content plays a secondary rôle. That this movement of water and electrolyte into the circulation is a function of the vaccine injection rather than of the saline hypodermoclysis alone is suggested by the work of Bogert, Underhill and Mendel (17) and of Smith and Mendel (18), who found that an amount of isotonic NaCl or Ringer's solution equal to the calculated blood volume, when rapidly injected intravenously into rabbits, has left the blood stream within 35 or 40 minutes; and also by the experiments of Underhill and Weinstein (19) who, after giving relatively large amounts of normal salt solution to rabbits either by mouth, subcutaneously, or intraperitoneally, were unable to detect any evidence of resulting change in blood volume.

9. Composition of the fluid entering the circulation in response to fever

These measurements afford the data necessary for calculating the composition of the fluid entering the blood stream during such alterations of fluid and electrolyte distribution as we have been studying. Table XV gives these calculations, expressed in the usual way as cubic centimeters of n/10 base, chloride, bicarbonate, and undetermined acid fraction, per 100 cc. of fluid, together with the number of cubic centimeters of such fluid entering the blood stream per kilogram of body-weight. It must be pointed out in this connection, however, that the errors of the figures are very large. First, the experimental error of a single plasma volume determination by the method given is probably close to 5 per cent. The error of a difference between two such deter-

minations on the same animal is therefore close to 7 per cent. The same applies, though to a lesser degree, to determinations of serum electrolyte concentration; but the figures for plasma electrolyte content, obtained by multiplying the serum electrolyte concentration by the plasma volume, must have an error from this source alone of more than 7 per cent. Secondly, there are the errors of individual variation in small series of cases, represented in this as well as in the other tables by the standard error. Finally, the figures for the percentage composition of the added fluid involve the error of a quotient, since they are arrived at by dividing the net accretion of electrolyte, in terms of cubic centimeters of n/10 solution, by the total added plasma, and

TABLE XV

Composition of added fluid

(The minus sign before a figure denotes the amount of that substance which has left the circulation and been replaced by another component.)

EXPERIMENTAL GROUP	AMOUNT PER KILOGRAM	BASE PER 100 CC. FLUID	CHLORIDE PER 100 CC. FLUID	BICARBONATE PER 100 CC. FLUID	UNDETERMINED ACID PER 100 CC. FLUID
	cc.	*cc. n/10*	*cc. n/10*	*cc. n/10*	*cc. n/10*
Shiga vaccine intraperitoneally	4.7	136	125	−17	28
B. coli vaccine intraperitoneally	5.3	309	169	−92	232
B. coli vaccine intravenously	10.4	165	118	−16	63
Radiant heat	7.0	90	55	−18	53
Shiga vaccine intraperitoneally with subcutaneous salt solution	16.7	124	130	6	−12

multiplying by 100 to show the electrolyte composition per 100 cc. The minus sign before a figure in Table 15 shows that that amount of fluid or electrolyte was removed from the blood stream.

The figures for the group receiving vaccine intravenously are of more value than the others, since the changes here were the most consistent. It is of interest that the average figures for the composition of added fluid in this case approximate closely the inorganic composition of spinal fluid, which some authors have cited as a fair representative of interstitial fluid under physiological conditions. In other groups, notably in the experiments in which radiant heat was applied and in which intraperitoneal vaccine was combined with subcutaneous administration of normal salt solution, the added fluid is seen to be more

dilute in point of view of its electrolyte concentration than normal plasma or spinal fluid, and also more dilute than the salt solution used for subcutaneous injection, which had a concentration of 154 cc. n/10 base and chloride per 100 cc. of solution. Thus, going back to the starting-point of these experiments, the finding of a depression of serum total base concentration following injection of vaccine, it has been demonstrated that this phenomenon depends on blood dilution.

TABLE XVI

Blood, plasma, and cell volumes per kilogram of body-weight in normal rabbits

RABBIT NUMBER	BLOOD VOLUME PER KILOGRAM	PLASMA VOLUME PER KILOGRAM	CELL VOLUME PER KILOGRAM	WEIGHT
	cc.	*cc.*	*cc.*	*grams*
101	57.0	39.0	18.0	2,140
102	48.2	32.7	15.5	2,370
103	48.4	31.6	16.8	2,150
116	47.1	32.6	14.5	1,870
117	49.3	35.5	13.8	2,270
123	57.3	38.7	18.6	1,240
124	52.7	34.5	18.2	1,205
125	66.6	44.7	21.9	760
132	64.8	40.6	24.2	1,590
133	55.8	34.5	21.3	1,740
134	50.2	29.1	21.1	2,220
135	52.9	32.6	20.3	2,070
Mean	54.2±1.8	35.5±1.2	18.7±0.9	
Standard Deviation	6.1±1.2	4.2±0.9	3.1±0.6	
Coefficient of Variation	11.3±2.3	12.0±2.4	16.4±3.3	

10. Total blood, plasma, and cell volumes in normal rabbits

In the course of these studies we have obtained data on the blood, plasma, and cell volumes per kilogram of body-weight in normal rabbits not previously subjected to any experimental procedure. The results are presented in Table XVI, and will be seen to agree well with those previously published by investigators who have used the carbon monoxide method: Douglas (7), 53 cc. of blood per kilogram; Boycott and Douglas (9), 51.2 cc. for male rabbits, 55.1 cc. for females, general average 53.7 cc. per kilogram; Salvesen (15), 50.3 cc. for male rabbits,

TABLE XVII

Total fixed base, chloride, bicarbonate, and undetermined acids per 100 cc. of serum, and total circulating plasma electrolytes per kilogram of body-weight, in normal rabbits

The figures represent cubic centimeters of n/10 solution

RABBIT NUMBER	TOTAL FIXED BASE PER 100 CC. SERUM	CHLORIDE PER 100 CC. SERUM	BICARBONATE PER 100 CC. SERUM	UNDETERMINED ACID PER 100 CC. SERUM	TOTAL FIXED BASE PER KILOGRAM	CHLORIDE PER KILOGRAM	BICARBONATE PER KILOGRAM	UNDETERMINED ACID PER KILOGRAM
101	177	111	15.5	50.5	69.1	43.3	6.0	19.7
102	167	107	26.3	33.7	54.6	35.0	8.6	11.1
103	178	116	14.9	47.1	56.3	36.7	4.7	14.9
116	167	108	16.8	42.2	54.5	35.2	5.5	13.7
117	174	105	21.2	47.8	61.7	37.2	7.5	17.0
123	174	112	23.5	38.5	67.4	43.4	9.1	14.8
124	172	103	24.1	44.9	59.3	35.5	8.3	15.4
125	171	101	24.7	45.3	76.4	45.1	11.1	20.3
132	174	110	22.2	41.8	70.6	44.7	9.0	16.9
133	166	107	20.6	38.4	57.2	36.9	7.1	13.2
134	178	100	27.4	50.6	51.7	29.1	8.0	14.7
135	156	97	28.0	31.0	50.9	31.7	9.1	10.1
Mean	171±1.8	106±1.5	22.1±1.2	42.7±1.7	60.8±2.3	37.8±1.4	7.8±0.5	15.2±0.8
Standard Deviation	6.1±1.2	5.3±1.1	4.3±0.9	6.0±1.2	7.9±1.6	5.0±1.0	1.7±0.4	2.9±0.6
Coefficient of Variation	3.6±0.7	5.0±1.0	19.4±4.0	14.1±2.9	13.0±2.7	13.2±2.7	22±4	19.2±3.9

49.8 cc. for females, general average 50.1 cc. per kilogram. They agree also with the results of Meek and Gasser (8) obtained by the use of the acacia method: 51.8 cc. per kilogram. On the other hand, they are appreciably higher than those of Utheim (20), who used the dye method (48.0 cc. per kilogram), and lower than those of McQuarrie and Davis (21) with their modification of the acacia method (64.9 cc. per kilogram); and much lower than those of Went and Drinker (14), who used a modification of the dye method (78 cc. per kilogram).

11. Total circulating plasma electrolyte in normal rabbits

In like manner we have tabulated the serum concentration of electrolytes in these animals and their plasma content per kilogram of body-weight. These are given in Table XVII, expressed in cubic centimeters of n/10 solution per 100 cc. or in milliequivalents per liter.

From the similarity of the coefficients of variation of plasma volume per kilogram and total circulating fixed base or chloride per kilogram it is clear that one has as much right to predict the plasma electrolyte content of an individual animal, in respect to base and chloride, as its total plasma volume, and that such predictions are governed by similar reservations.

DISCUSSION

The retention of water during fever by the organism as a whole has been known for many years and demonstrated in a wide variety of conditions. That at least part of this retained fluid may remain in circulation and bring about an increase in blood volume was suggested by the studies of Oppenheimer and Reiss (22) in patients with scarlet fever, and of Sandelowsky (23) in pneumonia. Barbour and others (24), by following the changes in hemoglobin concentration after submitting their subjects to a warm, moist atmosphere in which the cooling processes of the body were at a disadvantage, obtained evidence of blood dilution by a fluid rich enough in total solids to keep the latter figure for whole blood nearly constant; and in dogs they were able to show (25) that the circulating cells were diluted by a fluid of composition akin to normal plasma. More direct evidence of an increase in blood volume resulting from a change of atmospheric temperature was furnished by Barcroft and his associates (26), though parallel

observations of body temperature were not made. Soule, Buckman and Darrow (1) were able to show that fever brought about a slight to moderate increase in the plasma volume, and attributed this effect to the elevation of the metabolic rate with a resultant increase in the osmotic pressure of venous blood. Eppinger and Schürmeyer (27) determined the blood volume of patients with the carbon monoxide method, then warmed them with a lamp and repeated the determination; in seven such experiments the average change was an increase from 55 to 77 cc. per kilogram. There is some reason to doubt the validity of the 40 per cent increase so produced, on the technical grounds that they used a mixing time of only 5 to 7 minutes, which, in the case of the preliminary determinations, may well have been too short to permit as complete mixing of the blood as would have occurred in the same time under the conditions of vasodilation and accelerated blood flow brought about by the warming effect of the lamp; the result would be an abnormally low figure for the preliminary blood volume. However, such criticism refers only to the magnitude of the demonstrated change, not to its direction. They also recorded a 15 per cent increase in blood volume in a patient during a rise of temperature to 40.5°C. in malaria.

We believe that the increases in blood volume recorded in our own experiments, in which fever has been produced in a variety of ways, are accurate within the limit of error of the determination as carried out under afebrile conditions. A comparison of the mixing time of the blood in adults (28) and infants (12) when carbon monoxide is administered suggests that complete mixing is reached more rapidly in small subjects. It is probable, then, that the ten-minute re-breathing period used in these determinations on rabbits is fully long enough to obviate the objection which we have just raised on these grounds to Eppinger's results. Variations in the oxygen pressure of the air breathed were corrected for by analysis of the residual air for unabsorbed carbon monoxide. Changes in the form of the carbon monoxide hemoglobin dissociation curve resulting from alterations of blood pH would fall within the general error of the method. The objections which Lamson (29) has raised to blood volume determinations in general on the grounds of the unpredictable behavior of the liver as a reservoir of plasma, in response to injection or secretion of epinephrin, do not apply in this case, since he was unable to demonstrate this mechanism in rabbits.

As will be seen on comparison of the temperature records of Figures 4 and 6 with the blood volume changes of Tables VI and XII, only the roughest parallelism could be demonstrated between the degree of fever and the amount of change in blood volume. The technical limitations of the blood volume method used in our experiments—the fact that the determination requires at least 15 minutes' time and that it cannot be repeated at short intervals, say every five minutes—has prevented us, unfortunately, from exploring more minutely the relationship between vaccine injection and changes of blood and plasma volume. It would be of the greatest interest to know, for example, the order of events following a vaccine injection: to find out whether, and by what interval, a rise of temperature precedes the increase in blood volume. A change of blood volume following the application of radiant heat, without vaccine injection or other thermal agent, justifies us, perhaps, in inferring that, when vaccine is used, the change of blood volume is secondary and is directed toward temperature regulation. This is the argument advanced by Barbour and his colleagues (24) in explaining blood dilution, and by inference an increase of blood volume, as a means of augmenting the loss of heat through radiation. It is not likely that a close parallelism exists between the degree of fever and the change in blood volume, but rather that elevation of the body temperature sets in motion certain physiological reactions—among which increased blood volume (these experiments) and blood flow (Barcroft and Marshall (30)) are included—directed toward the reduction of fever; so that at any one moment the body temperature represents the balance of the two opposed forces. Whether an opposite change is brought out by chilling has not been determined. We are unable to say, also, whether changes of blood volume persist in prolonged attacks of fever.

Alteration of serum or plasma electrolyte concentration in infections accompanied by fever has been repatedly demonstrated. The most constant finding has been a lowering of chloride. Bicarbonate is usually decreased, though not invariably so. An increase of organic acids has been shown both indirectly and by direct determination. All of these features are sufficiently familiar not to require further comment here. There has been much speculation as to whether these changes represent blood dilution or a migration of electrolytes out of the circulation.

Binger and his associates (21) have only recently stated that the suggestion that the reduction in chlorides (in pneumonia) is the result of blood dilution awaits experimental proof.

In our own experiments, in which parallel determinations of both plasma volume and serum electrolyte concentration enable us to gain information about changes in total plasma electrolyte content, it has been possible to show that the parenteral injection of bacterial vaccine, giving rise to fever, or the induction of fever by irradiation of the animal with heat rays, brings about an influx of water and electrolytes into the circulation. Although the water intake of the animals was not controlled in all the experiments, in those in which radiant heat was used as the calorigenic factor it could be definitely demonstrated that this increase in fluid content of the circulating plasma took place without dependence on intake from outside sources. Under these circumstances, cessation of secretion and excretion may cause water to accumulate in the blood; and it is well known that with a rise in body temperature in man there occurs over a short time-interval a diminution or even a complete shut-down of sweating, of gastric secretion and of urinary excretion. In the rabbit, sweating is not a significant factor; concerning the other types of fluid output mentioned we have no definite information. Another possible mechanism of increase in plasma water content is an influx of fluid from reservoirs of intercellular fluid. Direct investigation of the part which this mechanism plays is difficult, but we have evidence of two kinds tending to show that it is significant. In the first place, the calculations of the composition of the water and electrolyte mixture by which the circulating plasma is augmented during fever are consistent with the thesis that this mixture is drawn from interstitial fluid reservoirs, even though the variation in the figures for single determinations does not justify one in drawing definite conclusions as to the composition of the mixture. In the second place, the experiment in which a subcutaneous injection of normal salt solution was given to increase the content of one of the main fluid reservoirs, the subcutaneous tissue, yielded the largest increase in plasma volume per kilogram of body-weight for the entire series of experiments.

Since diminution of serum chloride concentration in response to fever occurred in only one group of the series of experiments in which fever was produced, namely, in the group of animals subjected to radiant

heat, the series as a whole cannot be regarded as offering strong evidence that this phenomenon depends on plasma dilution. In that particular group, however, where the lowering of serum chloride concentration averaged about 7 per cent for the three animals in the group, the conclusion appears inevitable that this was brought about by a dilution of the blood with a fluid of lower chloride concentration than normal plasma.

SUMMARY

By simultaneous determination in rabbits of blood and plasma volume and of the serum concentration of total fixed base, chloride, and bicarbonate, it has been possible to study the movement of water and electrolytes into and out of the circulation in response to elevations of body temperature. Fever was produced in a variety of ways—by intraperitoneal injection of two different vaccines, by intravenous injection of a vaccine, and by irradiation of the animals with an electric lamp—and with all the methods an increase in circulating blood volume was brought about, varying only in degree roughly parallel to the change in temperature. Fever caused essentially no constant immediate change in circulating cell volume; the increase pertained to the plasma fraction. It caused invariably a diminution in the serum concentration of bicarbonate, usually a diminution of the concentration of total base, and sometimes a diminution but more often an elevation of serum chloride concentration. The increase in plasma volume, however, represented an influx into circulation of water and electrolyte of such degree that the total amount of circulating electrolytes was increased over the level existing prior to the elevation of body temperature. Usually, the total amount of circulating bicarbonate was diminished during fever, but this was not invariably the case. Calculations of the composition of the fluid entering the circulation at these times suggested that it was drawn from reservoirs of intercellular fluid, and this hypothesis was strengthened by the vigorous response of one animal in which the store of subcutaneous fluid had been reinforced by hypodermoclysis of normal salt solution. These calculations also suggested that the fluid entering the circulation might at times have a concentration of electrolytes lower than that of normal plasma or intercellular fluid, which may be offered as a possible explanation of the lowering of

plasma chloride concentration often found in pneumonia and other infections. The data obtained afford an approximate estimate of normal standards of total circulating plasma base, chloride, and bicarbonate in rabbits.

REFERENCES

(1) SOULE, H. C., BUCKMAN, T. E., AND D. C. DARROW: Blood Volume in Fever. *J. Clin. Investig.* **5**, 229 (Feb. 20) 1928.

(2) KEITH, N. M., ROWNTREE, L. G., AND J. T. GERAGHTY: A Method for the Determination of Plasma and Blood Volume. *Arch. Int. Med.*, **16**, 547 (Oct.) 1915.

(3) SMITH, M.: The Determination of Chlorides in Trichloracetic Acid Filtrates from Whole Blood and Plasma. *J. Biol. Chem.*, **45**, 437, 1921.

(4) FISKE, C. H., AND S. S. SOKHEY: Ammonia and Fixed Base Excretion after the Administration of Acid by Various Paths. *Ibid.*, **63**, 309, 1925.

(5) VAN SLYKE, D. D., AND J. M. NEILL: The Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement. I. *Ibid.*, **61**, 523, 1924.

(6) FISKE, C. H.: A Method for the Estimation of Total Base in Urine. *Ibid.*, **51**, 55, 1922.

(7) DOUGLAS, C. G.: A Method for the Determination of the Volume of Blood in Animals. *J. Physiol.*, **33**, 493, 1905–1906.

(8) MEEK, W. J., AND H. S. GASSER: Blood Volume. A Method for Its Determination with Data for Dogs, Cats and Rabbits. *Am. J. Physiol.*, **47**, 302, 1918–1919.

(9) BOYCOTT, A. E., AND C. G. DOUGLAS: On the Carbon Monoxide Method of Determining the Total Oxygen Capacity and Volume of Blood in Animals, with some Experiments on Anemia and Transfusion. *J. Pathol. & Bacteriol.*, **13**, 256, 1909.

(10) CARRIER, E. B., LEE, F. W., AND G. H. WHIPPLE: Determination of Plasma and Hemoglobin Volumes after Unit Hemorrhages under Controlled Experimental Conditions. *Am. J. Physiol.*, **61**, 138, 1922.

(11) VAN SLYKE, D. D., AND F. S. ROBSCHEIT-ROBBINS: The Gasometric Determination of Small Amounts of Carbon Monoxide in Blood, and Its Application to Blood Volume Studies. *J. Biol. Chem.*, **72**, 39, 1927.

(12) McINTOSH, R.: The Determination of the Circulating Blood Volume in Infants by the Carbon Monoxide Method. *J. Clin. Invest.*, **7**, 203, 1929.

(13) VAN ALLEN, C. M.: An Hematocrit Method. *J. A. M. A.*, **84**, 202 (Jan. 17) 1925.

(14) WENT, S., AND C. K. DRINKER: A Micromethod for Determination of the Absolute Blood Volume, with Data upon the Blood Volume of the Guinea-Pig, White Rat, Rabbit and Cat. *Am. J. Physiol.*, **88**, 468, 1929.

(15) SALVESEN, H. A.: The Determination of Blood Volume by the Carbon Monoxide Method. *J. Biol. Chem.*, **40**, 109, 1919.

(16) BARCROFT, J., HARRIS, H. A., ORAHOVATS, D., AND R. WEISS: A Contribution to the Physiology of the Spleen. *J. Physiol.*, **60**, 443, 1925.

(17) BOGERT, L. J., UNDERHILL, F. P., AND L. B. MENDEL: The Regulation of the Blood Volume after Injections of Saline Solutions. Studies of the Permeability of Cellular Membranes. I. *Am. J. Physiol.*, **41**, 189, 1916.

(18) SMITH, A. H., AND L. B. MENDEL: The Adjustment of Blood Volume after Injection of Isotonic Solutions of Varied Composition. *Am. J. Physiol.*, **53**, 323, 1920.

(19) UNDERHILL, F. P., AND H. W. WEINSTEIN,: The Rate of Absorption of Fluids by Different Routes. *J. Metabol. Res.*, **4**, 543, 1923.

(20) UTHEIM, K.: A Study of the Blood and Its Circulation in Normal Infants and Infants Suffering from Chronic Nutritional Disturbances. *Am. J. Dis. Child.*, **20**, 366, 1920.

(21) MCQUARRIE, I., AND N. DAVIS: Blood Volume Studies: IV. Blood Volume as Determined by the Change in Refractivity of the Serum Non-Protein Fraction after Injection of Certain Colloids into the Circulation. *Am. J. Physiol.*, **51**, 257, 1920.

(22) OPPENHEIMER, S., AND E. REISS: Untersuchungen der Blutkonzentration bei Scharlach mit besonderer Berücksichtigung der Nephritis. *Deutsch. Arch. f. klin. Med.*, **96**, 464, 1909.

(23) SANDELOWSKY, J.: Blutkonzentration bei Pneumonie. *Ibid.*, **96**, 445, 1909.

(24) BARBOUR, H. G., LOOMIS, N. M., FRANKMAN, R. W., AND J. H. WARNER: Heat Regulation and Water Exchange. V. The Phase of Blood Dilution in Man. *J. Physiol.*, **59**, 300, 1924–1925.

(25) HAMILTON, W. F., BARBOUR, H. G., AND N. M. LOOMIS: Heat Regulation and Water Exchange. VI. The Mobilization of Water and Salt in Warm Environments. *J. Physiol.*, **59**, 306, 1924–1925.

(26) BARCROFT, J., BINGER, C. A. L., BOCK, A. V., DOGGART, J. H., FORBES, H. S., HARROP, G. A., MEAKINS, J. C., AND A. C. REDFIELD: Observations upon the Effect of High Altitude on the Physiological Processes of the Human Body, carried out in the Peruvian Andes, chiefly at Cerro de Pasco. *Phil. Trans. Roy. Soc. London*, Series B, **211**, 351, 1923.

(27) EPPINGER, H., AND A. SCHÜRMEYER: Über den Kollaps und analoge Zustände. *Klin. Wchnschr.*, **7**, 777, (Apr. 22) 1928.

(28) CHANG, H. C., AND G. A. HARROP: The Determination of the Circulating Blood Volume with Carbon Monoxide. *J. Clin. Investig.*, **5**, 393, 1928.

(29) LAMSON, P. D.: The Part Played by the Liver in the Regulation of Blood Volume and Red Corpuscle Concentration in Acute Physiological Conditions. *J. Pharmacol. & Exper. Therap.*, **16**, 125, 1920.

(30) BARCROFT, J., AND E. K. MARSHALL, JR.: Note on the Effect of External Temperature on the Circulation in Man. *J. Physiol.*, **58**, 145, 1923–1924.

(31) BINGER, C. A. L., CHRISTIE, R. V., DAVIS, J. S., JR., AND A. HILLER: Blood Chlorides in Conditions Associated with Pneumonia. *J. Exp. Med.*, **49**, 603, 1929.

RESPONSE OF BLOOD GUANIDINE BASE CONCENTRATION IN NORMAL INDIVIDUALS AND IN PATIENTS WITH LIVER INJURY TO THE INGESTION OF METHYL GUANIDINE SULPHATE[1]

READ McLANE ELLSWORTH

From the Department of Medicine, Johns Hopkins University

In the past, abnormal concentrations of guanidine in the blood have been reported in several conditions. In 1916 Paton and Findlay (1) and Burns and Sharpe (2), in a series of papers, described guanidine retention in the blood of dogs from which the parathyroid glands had been removed, and attributed the symptoms of tetany to the guanidine increase. Subsequently, many observers, among them Greenwald (3) and MacCallum (4), have discussed this problem and it now seems questionable if, with modern methods of determination, there is any demonstrable increase in blood guanidine even during active tetany. In 1925, Major and Weber (5) (6) reported high blood guanidine values in certain cases of hypertension. Many of their patients may have been suffering from renal disease not demonstrable by the tests in vogue at the time. Later, Major and Weber ascribed the guanidine increase in their cases to renal insufficiency (7). This has been corroborated by the author, who has found, in cases of renal injury, that the blood guanidine is usually elevated.

In 1928, Minot and Cutler (8) first directed attention to the possible participation of the liver in guanidine metabolism. They found that dogs poisoned with carbon tetrachloride showed diffuse liver damage, associated with a pronounced increase in blood guanidine bases.

During the following year, Ellsworth (9) observed cases of arsphenamine jaundice, a condition in which liver damage so frequently occurs. He found, during the course of the illness, an increase of blood guanidine base. With recovery the guanidine fell to normal. An increase in blood guanidine was not present in five cases of obstructive and

[1] Received for publication March 20, 1930.

hemolytic jaundice examined, but, on the other hand, was noted in several cases of Laennec cirrhosis (in which jaundice was slight or absent.)

Minot and Cutler (8) remarked, in attempting to produce experimental guanidine poisoning in dogs, that a relatively large dose, (150 to 200 mgms. per kilo), even when given subcutaneously, was necessary to produce a significant rise in the blood guanidine. It seemed interesting, therefore, to observe, in human subjects, the effect upon the blood guanidine concentration of a small amount of guanidine given by mouth. This was studied first, in normal individuals, and secondly, in patients with liver damage, in whom the blood guanidine was already slightly elevated.

EXPERIMENTAL

The procedure was as follows: All patients, both normal cases and those with liver injury, fasted during the period of the experiment. A sample of blood (15 cc.) was taken in oxalate for analysis and the individual then received 200 cc. of water and, in capsules, 200 mgms. of methyl guanidine sulphate (3 to 4 mgms. per kilo). Further blood samples were taken at one, two and four hours thereafter. They were analyzed by the method of Major and Weber (5) (6) given below.

METHOD OF DETERMINATION OF GUANIDINE

Fifteen cubic centimeters of whole blood were taken in oxalate and a protein-free filtrate made by the method of Folin-Wu. It is highly important that the filtrate should be protein-free. The usual precautions must be followed to ensure this:

(1) Before filtering the precipitation mixture is thoroughly shaken and allowed to stand at least 10 minutes.

(2) The filter papers moistened with the precipitation mixture and the first few cubic centimeters are re-filtered. Eighty cubic centimeters of filtrate are taken and 0.5 cc. 10 per cent NaOH added. One-half gram charcoal (Merck, purified by acid) is introduced and the flask shaken thoroughly. It is allowed to stand for 4 to 5 minutes and then filtered through a 9 cm. filter-paper. After draining 5 to 10 minutes, the paper is returned to the flask and 25 cc. acid alcohol (2 cc. N Cl to 100 cc. alcohol) are added. This is shaken, allowed to stand over

night (or at least 5 hours) and filtered. Twenty cubic centimeters of alcoholic filtrate are evaporated to dryness on a water-bath, care being taken not to char. The residue is taken up in 7 cc. of distilled water. Two cubic centimeters of the solution are taken off and set aside. One cubic centimeter nitroprusside reagent is added to the remaining 5 cc. It is centrifuged 2 minutes and read immediately in a colorimeter against a standard guanidine solution simultaneously prepared.

The standard guanidine is made immediately before the determination by diluting the stock guanidine solution 10 cc. to 1000 cc. with H_2O. The stock solution is made by dissolving 0.1542 gram guanidine carbonate in 100 cc. of 0.1 N HCl. It is preserved with chloroform. The standard solution contains 0.01 mgm. guanidine per cubic centimeter.

Standard tubes, set up with 2, 3, 4, 5, 6, 8 and 10 cc. of standard guanidine solution, are all made up to 10 cc. with distilled H_2O. Two cubic centimeter of nitro-prusside reagent are then added to each tube.

The nitro-prusside reagent consists of:

10 per cent sodium nitro-prusside	1
10 per cent $K_3FeC_6N_6$	1
10 per cent NaOH	1
Distilled H_2O	9

The nitro-prusside reagent is mixed freshly for each series of determinations, but must stand at least half an hour before being used. By that time it will have faded to a light yellow color.

The colorimeter reading must be made within 5 minutes after the addition of the nitro-prusside reagent to the unknown solution, because of the gradual development of some color due to creatinine. $\frac{20}{R} \times \frac{21.9 \text{ mgms. in standard}}{2}$ = guanidine bases in milligrams per 100 cc. of whole blood, where R is the reading of the unknown in the colorimeter. Creatine and creatinine may be determined, if desired. upon the 2 cc. of filtrate set aside, and the appropriate correction for creatine made. Guanidine bases minus (0.12 × Creatine) = *Guanidine* (corrected). The details of the chemistry underlying the method are described by Major and Weber (6) and by Minot and Cutler (8).

The readings, to ensure accuracy must be made against the standard that the unknown matches most closely. We have found that

TABLE I

Normal Individuals

TIME	BLOOD GUANIDINE BASE								
	1	2	3	4	5	6	7	8	9*
hours	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
0	0.46	0.50	0.58	0.37	0.49	0.60	0.49	0.47	0.43
1	0.49	0.52	0.56	0.36	0.51	0.63	0.47	0.43	
2	0.49	0.53	0.60	0.37	0.51	0.58	0.48	0.45	0.44
4	0.49	0.51	0.59	0.38	0.53	0.61	0.51	0.43	0.47

* 350 mgm. methyl guanidine sulphate subcutaneously.

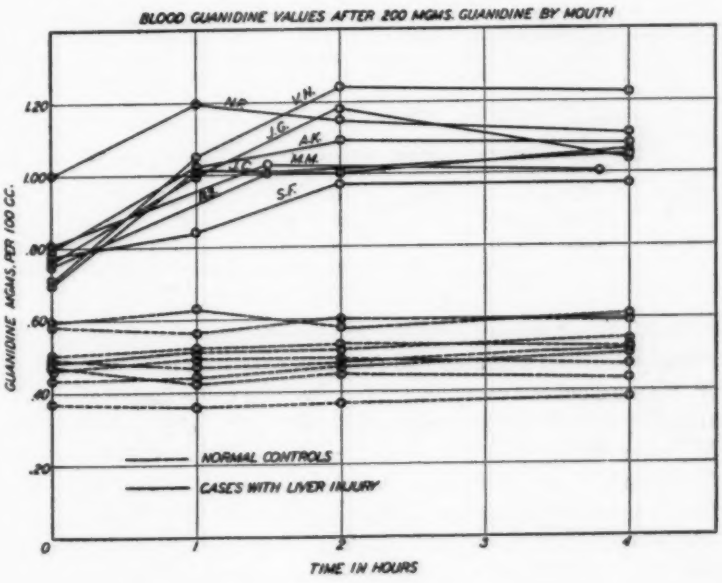

CHART 1

reading in a dark room with a blue light facilitates the matching of the yellow colors.

In the present paper corrections for creatine have not been made. The expression "blood guanidine" as used here indicates the concentration of guanidine bases giving the immediate color reaction with the nitro-prusside reagent.

This experiment was conducted first on eight individuals who were not, as far as could be determined, suffering from liver or kidney disease. In addition, one individual (R. E.) was given 350 mgms. of methyl guanidine sulphate subcutaneously. This was found to be very irritating around the point of injection and the procedure was not repeated. The results are shown in Table I and Chart 1.

Eight cases of liver injury were studied. Five of these patients were suffering from Laennec's cirrhosis, and in three of them (J. C., A. K., V. N.) at subsequent operation (omentopexy) the diagnosis was confirmed. Two probably had syphilis of the liver. In one of these (P. S.) the diagnosis of syphilis of the liver was made during an exploratory laparotomy on the Surgical Service. The eighth patient (M. M.) was suffering from diffuse carcinoma of the liver. In two patients (P. S. and M. M.) only three samples of blood were obtained—one, before; a second, at one and one half hours; a third, at four hours after administration of the guanidine. The results are shown in Table II and Chart 1. None of the individuals experienced any disagreeable sensation following the drug.

TABLE II

Cirrhosis of liver

TIME	BLOOD GUANIDINE BASE							
	Patient							
	V. N.	A. K.	J. C.	N. P.	S. F.	J. G.	P. S.	M. M.
hours	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
0	0.71	0.79	0.73	1.00	0.77	0.76	0.76	0.81
1	1.05	1.02	1.02	1.20	0.84	1.00		
2	1.24	1.09	1.00	1.14	0.97	1.18	1.00	1.02
4	1.22	1.08	1.06	1.10	0.97	1.03	1.05	1.00

RESULTS

In the eight control individuals, following the administration of guanidine by mouth, there was, in no case, any appreciable rise in

blood guanidine concentration. This is also true of the one case in which guanidine was given subcutaneously. The "curves" (Chart 1) are practically flat. This observation is in agreement with the experience of Minot and Cutler with dogs: *i.e.*, that small doses of guanidine are without effect upon the concentration of guanidine in the blood of the normal animal.

On the other hand, in the cases of liver injury, the initial values are somewhat above normal and have risen conspicuously at one hour, have increased still further at two hours and remain elevated at four hours after the administration of the guanidine. From these observations, it seems probable that, in order to demonstrate the effect, it is only necessary to take samples of blood before and two hours after administration of the guanidine unless a check be desired, when another blood sample may be taken at three or four hours after the ingestion.

These results we believe are significant. In none of the cases of cirrhosis selected was there demonstrable renal injury. It is interesting that in the patient M. M. a large abdominal mass of unknown origin was the presenting difficulty. There was a question whether or not the mass was in the liver. The bromsulphthalein test was entirely normal, but the guanidine curve was markedly abnormal. At operation, a diffuse carcinoma involving the whole liver was found. In the case, J. C., the x-ray examination revealed a "neoplasm of the colon," but because of the small liver with a hard, irregular edge and slight jaundice, cirrhosis was suspected. Again the guanidine curve was abnormal. The patient died and post-mortem examination revealed a syphilitic cirrhosis of the liver. The observations upon individuals with diffuse liver damage together with those of Minot and Cutler (8) on dogs, suggest that (in the absence of kidney disease), the finding of an elevated blood guanidine, which rises definitely after the administration of guanidine by mouth, may indicate diffuse liver injury.

It would be unwise to conclude at this time that the above observations warrant the use of this procedure as a test of liver function. In order to determine its exact significance it will be necessary to make numerous determinations upon various sorts and degrees of liver disturbance. Moreover, functional renal impairment must be rigorously excluded. Inasmuch as the opportunities for one observer are limited, the present results are published.

SUMMARY AND CONCLUSIONS

1. The administration of 200 mgms. of methyl guanidine sulphate by mouth did not produce in normal individuals a rise of blood guanidine base concentration in four hours.

2. In eight cases of diffuse liver injury, the initial blood guanidine base was slightly elevated and ingestion of the above amount of guanidine salt produced a conspicuous rise of guanidine in the blood.

3. Observations of blood guanidine base in the manner suggested may prove to be of assistance in detecting liver injury.

REFERENCES

(1) PATON, D. N., AND I. FINDLAY: *Quart. J. Exp. Physiol.*, **10**, 315, 1916. The Parathyroids: Tetania Parathyreopriva: Its Nature, Cause and relation to idiopathic tetany. Part IV. The Etiology of the condition and its relation to Guanidine and Methyl Guanidine intoxication.

(2) BURNS, D., AND I. S. SHARP: *Quart. J. Exp. Physiol.*, **10**, 345, 1916. The Parathyroids: Tetania Parathyreopriva: Its Nature, Cause and relations to idopathic tetany. Part V. Guanidine and Methyl Guanidine in the blood and urine in tetania parathyreopriva and in the urine in idiopathic tetany.

(3) GREENWALD: *J. Biol. Chem.*, **61**, 33, 1924. Is there a toxin in the blood of parathyroidectomized dogs?

(4) MACCALLUM, W. G.: *Medicine*. **3**, 137, 1924. Pathogenesis of Tetany.

(5) MAJOR, R. H., AND C. J. WEBER: *Bull. Johns Hopkins Hosp.*, **40**, 85, 1927. The probable presence of increased amounts of Guanidine in the blood of patients with Arterial Hypertension.

(6) MAJOR, R. H., AND C. J. WEBER: *Arch. Int. Med.*, **40**, 891, 1927. The Possible increase of guanidine in the Blood of certain persons with Hypertension.

(7) MAJOR, R. H.: *Am. J. Med. Sc.*, 176, 637, 1928. Renal Function in Arterial Hypertension.

(8) MINOT, A. S., AND J. I. CUTLER: *J. Clin. Investig.*, vi, 369, 1928. Guanidine retention and calcium reserve as antagonistic factors in carbon tetrachloride and chloroform poisoning.

(9) ELLSWORTH, R.: *Bull. Johns Hopkins Hosp.*, 1930 (April), Vol. xlvi. Blood guanidine in Normal Individuals and in Patients with Arsphenamine Jaundice.

DISAPPEARANCE OF DIABETES MELLITUS DURING THE DEVELOPMENT OF CIRRHOSIS OF THE LIVER[1]

JAMES BORDLEY III

From the Medical Clinic of the Johns Hopkins University and Hospital

In 1877 Claude Bernard (1), who was of course greatly interested in the rôle played by the liver in the production of diabetes mellitus, related the history of what seems an extraordinary case. A man 55 years of age developed, in the early months of 1873, general malaise, polyuria and polydipsia. His urine was found to contain 29 grams of sugar per litre. Under treatment he improved and the polyuria and polydipsia diminished, but at the end of 1873 his urine still contained sugar, 22 grams per litre. His condition remained about the same until the latter part of 1874 when weakness and emaciation made rapid progress and his abdomen began to swell. In January 1875, he presented the clinical picture of portal cirrhosis of the liver with massive ascites. At this time it was found that his glycosuria had cleared up and further examinations thereafter revealed the fact that the urine was completely sugar-free. In summarizing the case, Bernard remarks: "L'intérêt de cette observation réside dans la succession des phénomènes morbides: le malade était d'abord diabétique; il a plus tard une cirrhose du foie, et à mesure que la cirrhose se développe, le sucre disparaît des urines." It will be found that the interest in the following observation depends upon a similar succession of morbid phenomena.

REPORT OF CASE

G. S. (Unit no. 17,479) a Greek sea-captain, 47 years of age, was first admitted to the Medical Service of the Johns Hopkins Hospital, March 22, 1928, complaining of swelling of the abdomen. He was unmarried and had had little contact with his family in Greece, but so far as he could recall, none of his relatives had suffered from chronic illness. He was said, to have been a "sickly in-

[1] Submitted for publication April 22, 1930.

fant," but he himself did not remember having suffered from ill health prior to the onset of his present illness. From boyhood he had been a follower of the sea, had travelled in remote parts of the world and had led a vigorous life. He denied all infectious diseases. He had used various alcoholic drinks on occasion, but did not consider himself a heavy drinker and had "never been drunk." His dietary habits had been determined largely by the foods which were available on his long sea voyages. His appetite had always been excellent and he had not been subject to gastro-intestinal disturbances.

Present illness. Between 1918 and 1922, though he seemed quite well, his weight went down from 165 to 145 pounds. In December, 1922, he felt rather below par, noticed that he was drinking a lot of water and voiding frequently, and a good deal of the time was excessively drowsy. He realized that he had some bad teeth and thinking that these might be responsible for his trouble he consulted a dentist. The dentist detecting an acetone odor in his mouth advised him to go to a physician, who found his urine "loaded with sugar." A blood-sugar determination which was made at the time was said to have shown 480 mgms. He was placed upon a low carbohydrate diet and improved enough to return to sea. In December, 1923, medical advice was again sought and he was treated in the out-patient department of a New York hospital for several weeks without improvement in his glycosuria. In January, 1924, he was admitted to another New York hospital where, for almost three months, he was kept on a rigidly restricted diet. He responded slowly to treatment and left the hospital still showing a trace of sugar in his urine and with a fasting blood sugar of 130. He remained on shore until November, 1924, following his diet and urine all the while. Between November, 1924, and November, 1926, he continued to make sea voyages. He found it very difficult to adhere to his diet while on board ship; at the end of each trip the urine always contained large amounts of sugar which would diminish after a period of rest and dietary restriction. In the latter part of 1926 he began to be annoyed by excessive itching of his skin. During a sea trip which began in November, 1926, he lost weight and strength rapidly, and his legs became swollen. He landed in New York March 10, 1927, utterly exhausted. The following day he went to see Dr. L. G. Hadjapoulos who has been kind enough to furnish us with the following account of his condition at that time:

"On March 11, 1927, patient came to my office complaining of extreme weakness, itching of the skin more or less generalized, and with a diagnosis, that was made outside, of diabetes. His weight was 127 pounds, sugar in urine 4 plus, very heavy [no quantitative estimation was made]; blood-sugar was 360 mgms. He had a heavy acetone smell in the mouth and the urine was positive for acetone. He was immediately put under insulin treatment and practically starvation diet up to March 21, 1927, when the urine sugar was reduced to 2 plus, weight was 125½ pounds and there was a slight reduction in the cutaneous itching. Treatment was followed until April 5, 1927, when urine cleared from sugar and acetone, weight went up to 128½ pounds, and patient was feeling relatively comfortable.

"On April 8, 1927, patient saw me at the office, when for the first time I noticed a marked jaundice, more or less generalized but more marked on the face and arms. On this date he showed an enlargement of the liver, middle and left lobes, about 1½ inch below the costal margin, rather firm but not tender. As the urine and the blood sugar came down to normal figures (blood sugar 140 mgms.). I stopped the insulin treatment and increased the nitrogen intake."

Because of the progress of the jaundice the patient was admitted to a New York hospital May 11, 1927, under the care of Dr. Hadjapoulos who furnishes the following data which were gathered during the patient's two-months' stay in the hospital.

On admission the liver could be felt 3½ fingers below the costal margin, it was smooth throughout. The spleen edge was palpable on inspiration. The urine was negative for sugar, acetone and diacetic acid but showed albumin, a few hyaline casts and bile 1 plus. The stool showed bile, between a very faint trace and 1 plus. The blood-sugar was 190 mgms. and non-protein nitrogen 30 mgms. On admission the patient was running a slightly higher temperature than normal, but it was always below 100° for three weeks. On the fourth week, the temperature varied between 99° and 101°; on the fifth week between 99° and 101.5°; on the sixth and seventh weeks only axillary temperature was taken which was normal. The red blood cells varied between 3.5 and 4 million; haemoglobin between 65 and 75 per cent; leucocytes between 9 and 10 thousand. The Wassermann test was negative. Eye-grounds normal. The gall-bladder, visualized with kerasol, showed very little dye within the gall-bladder at the end of 12 to 15 hours. No negative shadows within the gall-bladder. After a meal the dye was expelled from the gall-bladder. Blood chemistry repeated on May 16 showed glucose 208 mgms. and non-protein nitrogen 23.5 mgms. Bromsulphthalein test for liver function showed no retention of the dye after half an hour. [Date of this test not stated.] On July 9, the Van den Bergh reaction was direct, 3 units. On July 16, just prior to discharge, the Van den Bergh was "delayed," 1.8 units, and the blood sugar was 160 mgms.

The patient left the hospital in July, 1927, improved but still weak and still slightly jaundiced. His condition had changed but little when on September 10, 1927, he again left port as captain of a ship. In November, 1927, the jaundice faded away, his strength improved and he noticed that his usual polyuria was diminishing. During the latter part of November he found that his abdomen was rapidly increasing in size. When he landed in Norfolk, Va., December 10, 1927, his abdomen was very tight and uncomfortable. He went immediately to a physician who performed paracentesis and removed "seven quarts of fluid." At this time the patient was very much surprised to find that, although he had been paying very little attention to his diet his urine was sugar-free and his blood-sugar was normal. After the paracentesis he felt well and strong and found that he was able to eat any sort of diet without spilling sugar in his urine and without developing any disagreeable diabetic symptoms. The ascites, however, recurred rapidly

and before the patient was admitted to the Johns Hopkins Hospital on March 22, 1928, his abdomen had been tapped six times, with intervals of about two weeks between the tappings.

Physical examination. (March 22, 1928.) Temperature 98.6°F. Pulse 80. Respirations 24.

A weather-beaten little Greek who had obviously lost considerable weight. He was very restless and complained of the tightness of his abdomen. The skin was dark brown in color. No jaundice. No spider angiomata. Complete adentia. Lungs clear throughout except for a few crackling râles at the bases. Heart slightly enlarged to percussion; second aortic sound ringing in character; soft systolic murmur at apex. Palpable peripheral arteries greatly thickened and tortuous, with readily visible pulsations. B.P. 140/78. The abdomen was large, protruding and obviously filled with fluid. There were no dilated veins over the abdomen or along the flanks. It was impossible to feel any of the abdominal viscera. Fingers showed slight clubbing. Rather marked pitting oedema over legs and lower trunk. Neurological examination disclosed no abnormality. Ophthalmoscopic examination revealed remarkable changes in both fundi which were about the same on the two sides. The disc margins were clearly outlined but were overlapped here and there by large patches of cream-colored exudate which blotted out most of the retinae. The retinal arteries were narrow and tortuous and there were a number of flame-shaped haemorrhages, some of them quite fresh.

Shortly after admission, the patient's discomfort was relieved by abdominal paracentesis which recovered 10,000 cc. of greenish-yelow, opalescent fluid. After the paracentesis the abdominal wall was relaxed and soft and it was possible to see and feel with ease a mass in the epigastrium and upper abdomen which from its location and general configuration was apparently the liver. Its lower border could be traced from just below the costal margin in the right anterior axillary line, across the abdomen about midway between the umbilicus and xiphoid, to pass again beneath the costal margin at the left mid-clavicular line. The lower edge of the mass was rounded, firm and somewhat irregular, but not definitely nodular. Its surface was quite smooth. No other masses were made out. The spleen and kidneys could not be felt.

Urine: Clear, amber. Specific gravity 1.020. Reaction acid. Sugar 0. Albumin ++. Microscopic: W.B.C. +, epithelial cells +, R.B.C. +. A few hyaline, granular and fatty casts. Diacetic acid, 0; Urobilin, 0; Bile, 0.

Blood: R.B.C. 3,450,000, Hgb. 70 per cent, W.B.C. 4,400. Smears showed a normal differential formula. The erythrocytes showed considerable anisocytosis and central pallor.

Ascitic fluid: 10,000 cc. greenish-yellow, opalescent fluid. Specific gravity 1.011. A few red blood corpuscles. Leucocytes: 480 per cu. mm. of which 42 per cent were mononuclear and 58 per cent polymorphonuclear cells. There was no bile or urobilin.

Blood Wassermann reaction negative.

Phthalein excretion 50 per cent in two hours.

Blood chemistry: N.P.N. 47 mgms. per cent; sugar 127 mgms. per cent. Van den Bergh reaction: indirect, 2 mgms. per cent.

Stool examination: Well formed stool of normal color and consistence. Bile present in normal amount.

TABLE I

DATE	FLUIDS		DIET				GLUCOSE	
	Intake	Output	Carbohydrate	Protein	Fat	Calories	Blood	Urine
1928	*cc.*	*cc.*					*mgms. per cent*	
April 5	2,025	1,385	199.8	79.7	150.2	2,542		0
April 6	3,200	2,330	200.1	80.0	149.6	2,539	148	0
April 7	3,200	1,955	199.9	80.5	149.5	2,539		0
April 8	2,850	2,530	200.2	80.4	150.1	2,546		0
April 9	3,550	2,865	250.4	99.9	180.0	3,110		0
April 10	2,835	2,110	250.6	100.3	180.4	3,115		0
April 11	2,955	1,810	249.7	99.7	179.3	3,100		0
April 12	2,750	2,260	249.8	100.1	180.3	3,111	125	0
April 13	3,485	1,875	250.2	100.2	180.4	3,114		0
April 14	2,430	2,540	300.0	99.8	199.9	3,498		0
April 15	3,550	1,730	299.9	100.6	200.1	3,502	117	0
April 16	3,325	1,970	360.1	110.0	198.8	3,776		0
April 17	3,000	2,505	360.2	110.1	199.8	3,771		0
April 18	3,130	2,080	360.0	109.7	199.7	3,782		0
April 19	3,900	2,405	360.2	109.2	200.1	3,784		0
April 20	3,475	2,650	359.7	110.1	200.0	3,786		0
April 21	3,775	3,150	299.9	100.6	200.1	3,502		0
April 22	3,000	2,575	360.0	110.0	200.0	3,787		0
April 23	4,000	1,200+	360.0	110.0	200.0	3,787		0
April 24	3,150	+	360.0	109.7	199.7	3,674		
April 25	3,000	+	360.3	110.0	199.9	3,678		
April 26	2,775	+	360.1	109.2	200.1	3,689		
April 27	3,650	+	361.3	110.3	?	?		
April 28		+					141	0

X-ray films of the chest and stomach showed no abnormality. There was very marked calcification of the vessels of the legs.

Basal metabolic rate: +13.

Course in hospital. The patient remained on the ward until April 28, 1928. During this period of more than five weeks he gained strength and there was no recurrence of the ascites. His weight remained practically stationary between 115 and 117 pounds. Most of our studies were directed toward determination of

his tolerance for carbohydrates. At the time of his admission to the hospital he was placed upon the regular "Ward Diet" which is relatively high in carbohydrate. After a period of five days on this diet he had failed to excrete any sugar in his urine and his fasting blood sugar was 135 mgms. per cent on March 27. Between March 28 and March 31, there was a remarkable and unexplained change in his condition which was characterized by abdominal discomfort, nausea and an elevation in temperature to 102.4°F. On March 28, a glucose tolerance test showed the following blood-sugar levels: Fasting 178 mgms.; one hour 217 mgms; two hours 218 mgms.; three hours 241 mgms. per cent. On the 29th and 30th the urine was sugar-free, but on the 31st he excreted 10.1 grams of glucose. On April 1, the urine was again sugar-free and thereafter throughout his hospital stay he showed no glycosuria. On April 5th he was placed in the metabolism ward where his diet was carefully calculated and his urine and blood were followed. Table I presents the results of this study. On April 15 we studied his response to adrenalin: At 8:35 a.m. his fasting blood-sugar was 117 mgms. per cent, pulse rate 76, blood pressure 130/83. 1.0 cc. of adrenalin 1:1000 was injected intramuscularly at 8:50 a.m. At 9:10 a.m. the blood-sugar had risen to 145 mgms. per cent, pulse 92, blood pressure 200/90. At 9:50 a.m. the blood-sugar was 180 mgms. per cent, pulse 100, blood pressure 120/78. A single determination of the blood lactic acid on March 28 showed 18.50 mgms. per cent, which was considered normal.

Course between hospital admissions. After leaving the hospital April 28, 1928, the patient experienced gradual improvement in his strength and felt that he was really getting well. He was inactive until November, 1928, when he captained a ship to England and back, which only served to convince him that he was not strong enough to continue sea-faring. In December, 1928, he was sick for a few days with chills, nausea and diarrhea. About January, 1929, he thought that his abdomen was beginning to swell again, but the swelling subsided spontaneously. In the summer of 1929, the swelling began again in earnest and between August 6th and December 26th, 1929, his abdomen was tapped nine times. (On November 28th a paracentesis trocar injured a blood vessel and he bled severely for about two and a half hours.) During this period he became progressively weaker, lost his appetite, thinks he was slightly jaundiced and was again annoyed by pruritus. When the abdominal swelling was at its maximum, his legs always became oedematous. January 7, 1930 he had a chill followed by prostration, dizziness, abdominal pain and diarrhea. He was readmitted to the hospital January 8, 1930.

Physical examination. (January 8, 1930.) When he arrived on the ward he looked quite ill and had obviously not profited by the long trip which brought him to the hospital. He seemed in partial shock with temperature 98°, pulse rapid, and blood pressure 86/64.

In general appearance he had not changed greatly since leaving the hospital. He was still emaciated, with a large, bulging abdomen. The skin had about the same brown shade. He had developed bilateral cataracts which interfered somewhat with his vision and made it impossible to see the optic fundi. The peripheral

arteries felt harder, and beading was a more conspicuous feature of the vessel walls. The heart was somewhat larger. There was considerable pitting oedema over the lower trunk and legs. There were no spider angiomata and no dilated veins over the abdomen but the umbilicus was blue in color and immediately around it there were a few small bluish veins. After 7,500 cc. of fluid had been removed from the abdomen, the liver margin could be felt as a firm, rounded, slightly uneven edge just below the costal margin.

Urine: Dark, cloudy. Reaction acid. Sugar +, albumin ++. Microscopic: R.B.C. few, W.B.C. +, hyaline casts ++, granular casts ++. Urobilin 0. Bile ++. Diacetic acid 0. (Following this first examination there was no glycosuria.)

Blood: R.B.C. 3,340,000 Hgb. 66 per cent W.B.C. 7,500.

Blood Chemistry: N.P.N. 58 mgms. per cent. Sugar 116 mgms. per cent, Van den Bergh reaction: delayed biphasia, 3.2 mgms. per cent.

Phthalein excretion: 45 per cent in 2 hours.

Following paracentesis and rest in bed he showed improvement. The blood pressure rose to 120/80 on January 11. With loss of ascites and oedema his weight fell from 60.5 kgms. to 50.5 kgms. On January 14 a glucose tolerance test, which was rendered somewhat unsatisfactory by the fact that the patient vomited 50 minutes after drinking the sugar mixture (80 grams glucose), showed the following results:

	Blood-sugar mgms. per cent
Fasting	109
½ hour	114
1 hour	122
2 hours	124
3 hours	120

January 21 a bromsulphthalein liver function test showed after 5 minutes a retention of 45 per cent of the dye, and after 30 minutes 15 per cent of the dye.

On January 23, because of the reaccumulation of the ascites with its attendent discomfort, the patient was transferred to the Surgical Service for exploratory laparotomy and omentopexy.

Dr. Dean Lewis explored the abdominal cavity through a right rectus incision. He found a hard nodular liver; no evidence of pancreatic disease. There were a number of adhesions between the viscera and in one place the omentum was attached to the anterior abdominal wall in such a way as to form what Dr. Lewis described as a "spontaneous Talma." The omentopexy was made more complete before the abdomen was closed.

The postoperative course was uneventful. In order to keep down the intra-abdominal pressure it was necessary to perform paracenteses on January 29 and February 3 and 13. Following the last paracentesis there seemed to be no tendency for the ascites to reaccumulate and the patient was allowed to get out of bed

and walk about the ward. On February 7 the blood-sugar was 95 mgms. per cent. Through the month of February, 1930, he ate a liberal carbohydrate diet without showing glycosuria except for scant traces of sugar in single specimens on February 15 and 17. On March 10 a sugar tolerance test after ingestion of 80 grams of glucose gave the following results:

	Blood-sugar mgms. per cent
Fasting	95
½ hour	160
1 hour	123
2 hours	110
3 hours	95

To summarize the case: A Greek sea-captain, now 49 years of age, began to lose weight in 1918, and in 1922 was found to have diabetes mellitus with acidosis. Between 1922 and 1927 he ran the up-and-down course of a severe diabetic on a poorly controlled diet. In March, 1927, he was observed during the course of acidosis which made rather slow response to rigidly restricted diet and insulin. In April, 1927, he became jaundiced and his liver was found to be enlarged. Jaundice continued for many months, but finally disappeared just before the onset of ascites in November, 1927. In December 1927, he first found that his diabetic symptoms had disappeared and that on an unrestricted diet there was no glycosuria and no hyperglycaemia. He was first seen by us in March, 1928, when he had outspoken evidences of portal cirrhosis of the liver. In April, 1928, on a carefully controlled high carbohydrate diet he showed no glycosuria and a blood-sugar only a trifle above normal. When seen again in the early months of 1930, the diagnosis of hepatic cirrhosis was confirmed by operation and throughout a long stay in the hospital he showed no evidences of diabetes mellitus.

DISCUSSION

Such cases as this, in which a long standing and severe diabetes clears up with development of cirrhosis of the liver, are apparently very rare.[2] Aside from Claude Bernard's case, mentioned above, I

[2] Dr. Elliott P. Joslin, who examined our patient on the ward in 1928, in his long experience with carefully followed diabetics had never seen a similar instance. Dr. Joslin subsequently went through his records with this matter in mind and was

have been able to find records of only two others in the literature both of which were reported by Benedict (2). In these cases the protocols are so brief that one finds it impossible to follow accurately the progress of events. Quincke (3) reported an interesting example of gradual diminution of glycosuria during the advanced ascitic stage of hepatic cirrhosis but in his case the urine did not become entirely sugar-free. Palma (4), Pusinelli (5), Naunyn (6) and others have recorded cases in which glycosuria disappeared in the terminal stages of cirrhosis. Lépine (7) says that he has followed several patients of this type but he associates the change in the urinary findings with the cachexia rather than with the specific liver disease.

When our patient was first studied in 1928, we rather hesitated to insist that he had cirrhosis of the liver, for it seemed difficult to believe that cirrhosis could be responsible for such a dramatic diabetic remission. At that time Wilder's case (8) of "hyperinsulinism" was in our minds and we wondered whether the patient might have a pancreatic tumor which had in some way compensated for his previous hyperglycaemia. The course of his illness between 1928 and 1930, and the final confirmation of our diagnosis by laparotomy, now give us sufficient evidence to say that the change in the progress of the diabetes was undoubtedly associated with a developing hepatic cirrhosis. Just what factor wrought this change we have no way of telling. It is apparent that the process which excited the diabetes either vanished or was overcome by some compensating mechanism which arose simultaneously with portal obstruction. Too little is known about the complex control of carbohydrate metabolism to allow us to entertain explanatory hypotheses.

---

kind enough to send us the histories of all of his patients in whom cirrhosis of the liver had been definitely established or seemed a likely possibility. There were in all sixteen such cases, excluding two examples of haemachromatosis. After a critical review of these records one does not feel justified in saying that the hepatic disease materially altered the diabetic course in any of them. In several of the cases the diabetes seemed rather less severe as the cirrhosis advanced, but in several others it became somewhat worse, and in not a single instance was an established diabetic followed into a definite remission with the onset of cirrhosis. (Dr. Joslin has already referred to our interest in this subject on pages 189 and 380 of the fourth edition of *The Treatment of Diabetes Mellitus*, 1928.)

SUMMARY

1. A case is reported in which a severe and long-standing diabetes mellitus cleared up as portal cirrhosis of the liver developed.

2. Attention is called to several similar cases reported in the literature.

3. Cases of this nature are apparently rare and the experience of others has shown that portal cirrhosis of the liver does not usually exert such an ameliorating effect upon diabetes mellitus.

REFERENCES

(1) BERNARD: Leçons sur le Diabète. Paris, 1877, p. 355.
(2) BENEDICT: *Arch. f. Verdauungskrankheiten*, **40,** 217, 1927.
(3) QUINCKE: *Berl. Klin. Wchnschr.*, **13,** 529, 1876.
(4) PALMA: *Ibid.*, **30,** 815, 1893.
(5) PUSINELLI: *Ibid.*, **33,** 739, 1896.
(6) NAUNYN: Der Diabetes Mellitus, 1906, S. 62–63.
(7) LÉPINE: Le diabète sucré. Paris, 1909, p. 427.
(8) WILDER *et al.*: *Jour. A. M. A.* **89,** 348, 1927.